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THE UNIVERSITY OF ALBERTA

DISINFECTION WITH SODIUM HYDROXIDE  
AND ITS APPLICATION TO MILK PRODUCTION

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR  
THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF DAIRY SCIENCE

by

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SEPTEMBER, 1961.



UNIVERSITY OF ALBERTA  
FACULTY OF GRADUATE STUDIES

The undersigned hereby certify that  
they have read and recommend to the  
Faculty of Graduate Studies for  
acceptance, a thesis entitled

DISINFECTION WITH SODIUM HYDROXIDE  
AND ITS APPLICATION TO MILK PRODUCTION

Submitted by R. L. Whitehouse in partial  
fulfilment of the requirements for the degree  
of Master of Science.

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## ABSTRACT

Immersion Cleaning, which is a method of cleaning and sanitizing milking machines by immersion in a 3% solution of caustic soda for the whole of the time between milkings, was developed in England for use with Direct-to-Can milking equipment, where the milk-contact-surface is reduced to a minimum and all of the equipment is treated by this method. Immersion Cleaning has been tried on farms in Canada using bucket machine milking plant, and equipment which was not able to be treated by this method, e.g. buckets, pails and other large items, was treated separately by another chemical method of cleansing.

The bactericidal efficiency of a disinfectant solution depends mainly on the concentration of the disinfectant, the temperature of the solution and the time of contact. In Immersion Cleaning, the immersion solution of 3% sodium hydroxide (which becomes diluted to approximately 2% at the end of a month's use) is used at the temperature of the milk-house where it is located, and the period of contact of the disinfectant solution with the milking equipment is the whole of the time between milkings. Disinfection studies with spores of Bacillus subtilis showed that the disinfectant properties of solutions of sodium hydroxide were markedly affected both by concentration of sodium hydroxide and by temperature in the ranges studied.



It was concluded that an immersion solution of an initial concentration of 3% sodium hydroxide might not produce a satisfactory bactericidal effect if the temperature at which the solution is used is low (around 30°F - 40°F). For farms where the temperature of the immersion solution is likely to be low during cold weather, an initial concentration of 5% sodium hydroxide is suggested.



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DISINFECTION WITH SODIUM HYDROXIDE  
AND ITS APPLICATION TO MILK PRODUCTION

GENERAL INTRODUCTION

The purpose of this work was to determine whether a process of cleaning and sanitizing milking utensils by immersion in sodium hydroxide solution between milkings would be satisfactory under Canadian climatic conditions. The process, called Immersion Cleaning, was developed in England in 1955 and was designed for use with one particular type of milking system known as Direct-to-Can milking. The process has been tested also with types of milking systems different from that for which it was designed.

In relation to this work, the disinfectant properties of sodium hydroxide solutions at different concentrations and at different temperatures has been investigated to see whether any change in the process of Immersion Cleaning is necessary to produce satisfactory bacteriological results under Canadian climatic conditions.







## GENERAL PRINCIPLES OF CHEMICAL DISINFECTION

A bacterial population of a single species or strain reproduces at a logarithmic rate when provided with a suitable environment and after a lag phase in which the organisms become adjusted to the ambient conditions. Then, when conditions become less favourable owing to lack of suitable nutrients, accumulation of waste products, overcrowding, etc., the rate is slowed down until the culture is in dynamic equilibrium and the concentration of viable organisms remains the same over a period of time, i.e. the rate of production of new viable cells equals the rate at which cells die off. Thereafter, the death rate increases and eventually assumes a logarithmic rate and finally this slows down progressively so that few organisms remain viable long after the bulk of the population has died. The time taken to reach a maximum population for a particular species or strain depends upon the conditions of temperature, presence of suitable nutrients, pH, lack of inhibitory substances, etc. peculiar to that organism. However, even during the logarithmic phase of growth where the growth rate is at its maximum, it has been reported that the percentage of viable organisms seldom rises above 90% (Wilson, 1922).

The normal growth and reproduction cycle may be adversely affected by chemicals which are either toxic directly or create conditions which are unfavourable to the normal functioning of the cells.

Bacteriostasis has been defined as a condition under which bacteria



reproduce at a rate less than normal (Berry & Parkinson, 1955). The conditions may be such that the cells are not directly killed but are prevented from multiplying and the cells then die of senescence. If the cells of a population are actively killed, this is termed bactericidal action. The difference between bacteriostasis and bactericidal action may be one of degree. When a cell population is subjected to a weak disinfectant action, the most sensitive cells are killed (bactericidal action), whereas less sensitive cells may be kept in a state of suspended animation (bacteriostatic action) while other more resistant cells may be relatively unaffected. That bacteriostasis and bactericidal action are matters of degree is still not generally accepted since many chemical substances may produce bacteriostasis but not exert a bactericidal action if the concentration is increased.

#### MODE OF ACTION OF CHEMICAL DISINFECTANTS

Enzymes and enzyme systems are the basis of all living things and are responsible for the complex chemical reactions which constitute metabolism and life. Enzymes are highly specific in their action, one particular enzyme catalyzing one or only very few steps in a biochemical reaction. Each enzyme requires exacting conditions under which to function and, if these conditions are altered slightly, the activity of the enzyme is greatly affected. Since enzymes are so highly specific, it seems logical to suppose that different enzyme systems are predominant in a bacterial cell at different times of its life cycle, e.g. during reproduction, the enzyme systems necessary for carrying out this process are in a higher concentration and are more active than they are



at other times. In cells of younger cultures, the enzymes necessary for metabolism would be more active and vital than in cells of older cultures, etc.

Enzymes are composed of a protein part and a non-protein, simpler part which is directly responsible for carrying out the chemical change the enzyme is catalyzing. Thus, being proteins, enzymes are susceptible to denaturation and it is by denaturation of enzyme proteins that it is thought many disinfectants act, at least in part. Many disinfectants act by blocking enzyme systems, i.e. stopping one particular step in a reaction and this may affect the normal functioning of the whole cell, particularly if the step interfered with is of a particularly vital pathway. Different enzyme systems differ in their sensitivity to different disinfectants.

In addition to affecting the enzymes of a cell, disinfectants acting by denaturation may, of course, affect other protein parts of the cell. Also structural damage to the cell may be a result of disinfection with certain disinfectants, and damage particularly to the cell wall and cell membrane may be responsible for, or contribute to, the death of the cell. Often, several effects are involved simultaneously, but most if not all disinfectants directly affect, by denaturation, chemical reaction with, or blocking, the essential enzymes of the cell.





## FACTORS AFFECTING CHEMICAL DISINFECTION

### TIME.

As the enzymic make-up of a cell changes during its life-cycle and as enzymes vary in their susceptibility to a particular disinfectant, so will individual cells vary in their susceptibility to a particular disinfectant. This may be part of the explanation that disinfectants in suitable concentrations do not produce an immediate kill of all cells, but individual cells vary in their susceptibility, some being killed quickly while other more resistant cells may survive considerably longer; disinfection is not usually immediate but is a gradual process. If chemical disinfection is concerned with interfering with enzymes inside the cell, penetration of the disinfectant to the site of action also takes time and so there is a time element even in the disinfection of the most susceptible cells.

### TEMPERATURE.

High temperature itself, particularly in a moist atmosphere, causes denaturation of proteins and enzymes and thus achieves disinfection. But also, when a chemical disinfectant is used, its activity is greatly influenced by temperature. A normal chemical reaction is usually increased by a factor of approximately 1 - 3 for an increase in temperature of  $10^{\circ}\text{C}$  (i.e. usual chemical reactions have a temperature coefficient, or  $Q_{10}$ , of 1 - 3). However, many reactions involving protein and enzyme denaturation are increased by a factor very much higher than normal chemical reactions. The  $Q_{10}$  may be as high as 300 if the temperature is increased by  $10^{\circ}\text{C}$  in a particular range over





which the reaction is effective. Thus the temperature at which a chemical disinfection is carried out is of great importance.

#### CONCENTRATION OF DISINFECTANT.

Generally, increasing the concentration of a disinfectant increases the rate of disinfection. There is a considerable difference between the effectiveness of different disinfectants, and a particular concentration of one disinfectant, of course, will not necessarily produce the same effect as a similar concentration of another. Similarly, increasing the concentration of one disinfectant by a particular factor (e.g. by doubling the concentration) will not necessarily produce the same effect as changing the concentration of another by the same degree, i.e. the concentration coefficients of different disinfectants vary. Withell (1938) expresses the concentration coefficient,  $n$ , in the following equation,

$$KC^n t = \log_e \frac{B}{b} \quad \text{where} \quad \begin{array}{ll} K = & \text{the time velocity constant} \\ & \text{independent of concentration.} \\ C = & \text{concentration.} \\ B = & \text{initial number of viable} \\ & \text{organisms.} \\ b = & \text{number viable after} \\ & \text{time, } t. \\ n = & \text{concentration coefficient.} \end{array}$$

If  $n = 1$ , and if the concentration is doubled, then  $C^n = 2^1$  and the time for killing the same number of organisms is halved. If  $n = 5$ , then doubling the concentration reduces the time for killing the same number of organisms by  $2^5$ , or 32 times.



## TYPE AND AGE OF ORGANISM.

Different species and strains of bacteria vary in their susceptibility to a particular disinfectant. One disinfectant may be very effective in killing one type of organism yet have little effect on other types. Generally, bacterial spores are the type of microbial life most resistant to disinfection, this usually being attributed to the resistant spore coat and to the lack of free water inside the cell. With disinfection by sodium hydroxide the next most resistant type is Gram-positive bacteria generally and in particular the staphylococci. Gram-negative organisms are relatively susceptible to sodium hydroxide. Younger, actively growing cultures are usually more sensitive to disinfection than older cultures. With most bacterial spores, maximum resistance is attained after a period of maturation (Curran, 1952).

## PRESENCE OF ORGANIC MATTER.

The presence of organic matter reduces the efficiency of a disinfectant. Since most disinfection processes involve reaction with protein materials in the bacterial cell, organic matter present in the medium competes with the organism for the disinfectant. Colloids or particles may adsorb the disinfectant and thus reduce its concentration in solution. Sykes (1958) points out that protection may also arise from coating the cells with organic matter which may at least delay the reaction and allow the cells to adapt themselves to resist. Sodium hydroxide is less affected by the presence of organic matter than are many other disinfectants. (McCulloch, 1945).



## EFFECT OF pH.

Highly acid or alkaline conditions alone may cause disinfection through enzyme denaturation, etc. The cations in the case of alkalis and the anions in the case of acids may also contribute to the disinfectant effect. Since most chemical reactions are affected by pH, chemical disinfection is influenced greatly by pH.

## EFFECT OF INITIAL CONCENTRATION OF ORGANISMS.

Many workers (e.g. Watkins & Winslow, 1932; Porter, 1946) have reported that when a higher concentration of organisms is exposed to disinfectant action, the total extinction time is prolonged. This is because with a higher concentration of organisms there are more cells which are resistant and the chances of there being cells of very high resistance is greater.

## OTHER FACTORS.

Disinfection will be affected by the state of dispersion of the micro-organisms in the disinfection solution, e.g. whether they are in the form of clumps (either by growing in such a form naturally or being caused to assume this form by contact with the disinfectant or by some other cause) or whether they are in a homogeneous suspension. If clumps exist in the bacterial population, those cells in the centres of the clumps will be partly protected by the outer cells from the action of the disinfectant and thus the population as a whole will have a greater survival time than if the cells occurred singly or not in clumps.



Similarly, if the cells are deposited onto a surface, especially in the presence of organic matter, they will be more resistant to disinfection. Where this is the case, detergent properties of the disinfectant are also important.

The main factors affecting disinfection have been given above. As this is such a complex process, all these factors must be considered in any study of chemical disinfection.







## APPLICATION OF CHEMICAL DISINFECTION TO THE DAIRY INDUSTRY

Since, in treating a milk-contact-surface with a chemical compound, there is a possibility of some of this compound being left to come into contact with the milk, the choice of compounds which can be used in sterilizing dairy equipment is limited to relatively non-toxic substances, such as sodium or calcium hypochlorite, sodium hydroxide, iodophores, quaternary ammonium compounds, etc.

From the previous section it can be realized that the efficient use of a chemical disinfectant depends on many factors. However, when correctly used, chemical disinfectants are very effective in producing satisfactory reduction in bacterial numbers and these substances have many advantages to commend their use in certain circumstances in the dairy industry over other methods of sterilization.

The main points to be observed in chemical disinfection are the use of the correct concentration of disinfectant at the correct temperature for the correct length of time. If any one of these factors is reduced, the disinfection treatment is adversely affected. Under practical conditions there is a tendency for the farmer to want to reduce on cost and time necessary for the 'washing-up' process and the time of contact of the equipment with the disinfectant may be cut short or the correct temperature not applied, etc. Thus, in devising a satisfactory routine cleansing process, the human element must be taken into consideration.



The work described here is concerned with methods of cleaning and 'sterilizing' farm milking equipment, therefore problems concerning milk-processing will not be considered.

The purpose of the 'washing-up' process is to remove soil and micro-organisms from milking equipment surfaces so that milk which comes into contact with the equipment is contaminated as little as possible. Also, of course, cleaning is necessary to maintain the milking equipment in a satisfactory working order.

Milk residues and dirt may at times be dried onto equipment and in order to remove these, a detergent must be used in the first place. When the surfaces are free from dirt, the organisms remaining on the surfaces may be destroyed by a sanitizing or sterilizing operation. The two operations of removal of dirt and sanitation may, however, be combined into a single operation.

One of the most efficient ways of sterilizing equipment is by the use of steam. But, the equipment for raising steam is expensive as is the cost of its operation, and this is a strong factor in deterring the small farmer from using steam. Steam sterilization, like chemical treatment, is dependent on several conditions, such as application of the correct temperature for the minimum time. Application of steam sterilization is used after the thorough removal of soil by a suitable detergent.

Milk-contact-surfaces are usually made of metal, glass or rubber. The majority of metal surfaces are now of stainless steel with some of



aluminium and, in limited cases of tinned steel. Both metal and glass surfaces are relatively easily cleaned and sanitized, but the rubber surface presents a problem.

Fat from the skin of the udder and teats of the cow and from the milk is able to penetrate rubber, altering its physical and chemical properties, and rendering it suitable for the entry of bacteria. Bacteria in the body of rubber equipment are protected during cleansing of the equipment unless the sanitizing treatment is able to penetrate the rubber, by the use of heat or a chemical substance able to penetrate the rubber. Such bacteria, if not destroyed, may be liberated at the next milking by the stretching and relaxing of the rubber in contact with the milk. As mentioned previously, heat can penetrate rubber and destroy these organisms, but chemical disinfectants, such as hypochlorites, iodophores, etc. are unable to do this and thus will leave the bacteria untouched.

If fat is allowed to accumulate in the rubberware, equipment such as teat-cup liners soon become distorted and swollen and lose their elasticity as a result of oxidation. If heat is used as the sanitizing agent, this loss of elasticity will be pronounced as heat enhances the rate of rubber oxidation due to absorbed fat (Gardner & Berridge, 1952; Cooper & Gardner, 1953).

It is a common practice to remove the fat from used liners either by periodic boiling in caustic soda or by using two sets of liners, keeping one set in soak in a 5% solution of caustic soda for two weeks while the other set is in use. In this way the useful life of the liner





is extended and in addition micro-organic growth which builds up in the pores of the liner is removed.

The main problems in the disinfection of milking equipment then are concerned with the rubber surface, particularly that of the teat-cup liners. In 1931 Parfitt suggested the use of a dilute solution of caustic soda for treating milking machine clusters between milkings. Caustic soda has the advantage of being both a good disinfectant and a good detergent, and is able to penetrate rubber, remove the fat and destroy organisms protected in the rubber by the fat. This method, which was developed by Johns (1933), became known as Wet Storage and the method consisted of filling the whole of the cluster assembly with a 0.5% solution of caustic soda and leaving between milkings. This treatment helped to reduce fat absorption and to keep the liners in a satisfactory bacteriological condition. However, if this method is used without a preliminary detergent wash, trouble may arise from a mineral deposit forming in the liners particularly in areas where the water supply is hard. Accordingly this method is usually recommended for use in conjunction with another method of cleansing to help preserve the rubber and maintain sanitary conditions of the teat-cup assembly.

A method of cleaning and sanitizing milking equipment, known as Immersion Cleaning, was devised in England in 1953 by Thiel, Clough & Clegg (1955). This method, which was designed to overcome problems created by the rubber surface and the personal factor, uses a 3% solution of caustic soda in which the milking equipment is allowed to remain between milkings. The only treatment which the equipment receives





before immersion is a rinse in cold or tepid water to remove milk residues and other soil. The same caustic soda solution is used for a month and thus attention to the daily or twice-daily preparation of the sterilizing solution by the farmer is eliminated. Observation of the correct temperature is removed since the solution operates at the temperature of the dairy or milk-house. The period of contact of the equipment with the disinfectant is long (a matter of hours) and even if cut short to some extent by the farmer, is still likely to be long enough to ensure adequate disinfection. But the farmer does not have to wait while the disinfection process is taking place and so it is not so likely that it will be cut short considerably as might be the case with sterilization with hypochlorite where the disinfection period is a matter of minutes. The method is extremely simple and easy to perform and requires little and inexpensive equipment. The cost of chemicals is also reduced.

In addition to producing satisfactory bacteriological results, the method greatly extends the useful life of the rubber parts by extraction of absorbed fat.

The method of Immersion Cleaning was developed for use in a temperate climate with direct-to-can milking equipment where the equipment coming into contact with milk is reduced to a minimum. For this study it was decided to investigate whether this system of treating milking utensils would work satisfactorily under conditions likely to be experienced in a Canadian winter and with different types of milking machines. Immersion Cleaning is used to the greatest



advantage with direct-to-can milking because of the small area of milk-contact-surface and because the whole of the milking equipment can be treated by immersion. There are two types of bucket milkers, the usual bucket type milking unit where the milk is conveyed from the teat-cup assembly to the bucket via a long milk-tube, and the suspended bucket type of machine where the inflations are attached directly to a lid which fits onto the bucket. Both types have been tested in this investigation. With bucket plant, buckets, carrying pails and milk strainers were cleansed in the usual way with detergent and sanitizer.

The second part of this work was devoted to studying the disinfectant properties of sodium hydroxide solutions at a range of concentrations and temperatures at which the immersion solution could be used. This was undertaken to ascertain whether any change was necessary when Immersion Cleaning was used under Canadian climatic conditions.



## PART I

### IMMERSION CLEANING TRIALS

#### INTRODUCTION

#### THE TECHNIQUE OF IMMERSION CLEANING AS USED FOR DIRECT-TO-CAN MILKING EQUIPMENT.

The method involves the complete immersion of all milk-contact-surfaces in a 3% solution of caustic soda for the whole of the time between milkings. After milking, the equipment is merely rinsed in water before immersion in caustic soda solution, and before milking merely rinsed free from caustic soda. Once a month the equipment is dismantled, brushed in hot detergent solution and the immersion solution changed.

The equipment is simple and consists of a mild steel bin which holds 12 gal of caustic soda solution when two-thirds full. The bin is fitted with a lid and contains a mild steel basket which is designed to accept the milking equipment. The basket (Fig. 1 and Plate 1) has a perforated base and a bridge on which the clusters hang, and a spiral rack to accept the milk tubes and air tubes.

Before milking, the metal basket is lifted free from the solution and lodged on the rim of the bin to allow the equipment to drain.



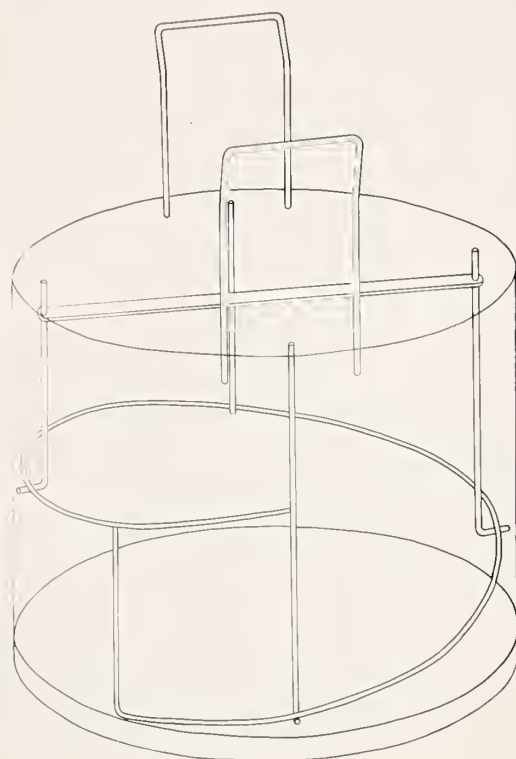


Fig. 1. The immersion basket illustrating the bridge on which the clusters hang and the spiral rack to accept the milk tubes and air tubes.







Plate 1. The immersion basket empty showing the bridge on which the clusters hang, the spiral rack to accept the milk tubes and air tubes, and the perforated base.



Then the basket is placed on the floor where the equipment may be hosed to free the outside from caustic soda. The equipment is then lifted by hand into a wash trough which contains 5 gal of water chlorinated at approximately 50 p/m. The disinfectant is added not to aid the sterilization of the equipment but to avoid recontamination of the equipment from the wash trough or water.

Adequate rinsing at this stage is not difficult and there is an automatic safeguard against inadequate rinsing because the rubber tubes will not remain on the metal parts of the machine during milking but will slip off if rinsing has been inadequate. The machines are then assembled and are ready for milking.

After milking, the equipment is brought back to the milk-house, and dung or any other dirt removed from the outside of the clusters by a wet brush. The equipment is then rinsed with water, or for economy with the same hypochlorite solution used before milking. The long milk tubes and air tubes are disconnected from the clawpieces and lids and, after rinsing, are coiled around the inside of the metal basket. The tubes are automatically positioned by the spiral rack to prevent air locks. The bungs are removed from the clawpieces and these together with other small parts are placed in the bottom of the basket. At this stage it is desirable to release the tension on the liners of the free standing bucket plant. Those liners which have a deep skirt can readily be kept free from the shell (as shown in Plate 2) though this skirt tends to augment carryover of caustic soda solution to the rinse trough. Freeing the liner from the end of the shell allows free rinsing between the shell and the liner. Otherwise caustic soda







Plate 2. Liners with deep skirts kept free from the shells as they are during immersion.



might find its way between the liner and the shell and eventually to the pulsator. With two-piece liners and liners with thin lips, it is desirable for the short milk tube to be manufactured with a lug on the outside in order that the shells can be kept free from the liners during immersion (Plate 3).

The clusters are then rinsed in the hypochlorite solution and lodged on the bar of the metal basket. The lids are then placed alongside the clusters so that they are supported vertically by the clusters and the sides of the basket (Plate 4). The specially designed lid (Thiel et al., 1955) consists of a simple stainless steel disk through which are welded two tubes, one vertical and the other at an angle (to avoid churning of the incoming milk by directing it onto the side of the can). On the underside of the lid are three studs which hold a rubber gasket loosely so that the cleaning solution and rinse water can reach all surfaces. It is desirable that all the milking equipment which is immersed is of stainless steel, as with plated equipment the plating is readily removed by the caustic soda solution. The caustic soda solution has no effect on the mild steel of the basket and bin.

Finally, the basket is lowered into the solution, the bin lid replaced and the equipment left thus until next milking. With an operator who has become accustomed to the procedure, this takes about 2-1/2 minutes before milking and 2-1/2 minutes after milking.

Once a month the equipment is dismantled and the milk-contact-surfaces brushed in a hot detergent solution. This is the only time at which the milk-contact-surfaces are brushed. At this time the

(1911)

The following table shows the results of the experiments conducted during the year 1911. The experiments were conducted in the laboratory of the Department of Agriculture, and the results are given in the following table. The table is divided into two parts, the first part showing the results of the experiments conducted in the laboratory, and the second part showing the results of the experiments conducted in the field. The results of the experiments conducted in the laboratory are given in the following table:

Experiment	Results
1. The effect of the temperature of the soil on the growth of the plant.	The results of the experiments conducted in the laboratory show that the growth of the plant is affected by the temperature of the soil. The growth is greatest when the temperature of the soil is between 60° and 70° F., and is least when the temperature of the soil is below 50° F. or above 80° F.
2. The effect of the amount of water on the growth of the plant.	The results of the experiments conducted in the laboratory show that the growth of the plant is affected by the amount of water. The growth is greatest when the amount of water is between 1 and 2 inches, and is least when the amount of water is below 1 inch or above 2 inches.
3. The effect of the amount of light on the growth of the plant.	The results of the experiments conducted in the laboratory show that the growth of the plant is affected by the amount of light. The growth is greatest when the amount of light is between 1 and 2 inches, and is least when the amount of light is below 1 inch or above 2 inches.
4. The effect of the amount of air on the growth of the plant.	The results of the experiments conducted in the laboratory show that the growth of the plant is affected by the amount of air. The growth is greatest when the amount of air is between 1 and 2 inches, and is least when the amount of air is below 1 inch or above 2 inches.
5. The effect of the amount of food on the growth of the plant.	The results of the experiments conducted in the laboratory show that the growth of the plant is affected by the amount of food. The growth is greatest when the amount of food is between 1 and 2 inches, and is least when the amount of food is below 1 inch or above 2 inches.

The results of the experiments conducted in the field are given in the following table:

Experiment	Results
1. The effect of the temperature of the soil on the growth of the plant.	The results of the experiments conducted in the field show that the growth of the plant is affected by the temperature of the soil. The growth is greatest when the temperature of the soil is between 60° and 70° F., and is least when the temperature of the soil is below 50° F. or above 80° F.
2. The effect of the amount of water on the growth of the plant.	The results of the experiments conducted in the field show that the growth of the plant is affected by the amount of water. The growth is greatest when the amount of water is between 1 and 2 inches, and is least when the amount of water is below 1 inch or above 2 inches.
3. The effect of the amount of light on the growth of the plant.	The results of the experiments conducted in the field show that the growth of the plant is affected by the amount of light. The growth is greatest when the amount of light is between 1 and 2 inches, and is least when the amount of light is below 1 inch or above 2 inches.
4. The effect of the amount of air on the growth of the plant.	The results of the experiments conducted in the field show that the growth of the plant is affected by the amount of air. The growth is greatest when the amount of air is between 1 and 2 inches, and is least when the amount of air is below 1 inch or above 2 inches.
5. The effect of the amount of food on the growth of the plant.	The results of the experiments conducted in the field show that the growth of the plant is affected by the amount of food. The growth is greatest when the amount of food is between 1 and 2 inches, and is least when the amount of food is below 1 inch or above 2 inches.





Plate 3. Two-piece liners and liners with thin lips showing the lugs on the stems to allow the liners to be kept free of the shells during immersion. (From Thiel, Clough & Clegg, 1955).





Plate 4. Milking equipment stacked in the immersion basket.



rubbers are inspected for accidental damage. The solution in the bin is discarded, any sediment in the bottom of the bin rinsed out and fresh solution prepared. In addition to caustic soda it has been found necessary to add a small quantity of ethylenediaminetetraacetic acid (EDTA), a sequestering agent, to prevent the accumulation of insoluble soaps resulting from the interaction of fat extracted by the caustic soda and minerals from milk residues and water hardness. The amount of EDTA added depends on the hardness of the water, 2 ozs. being added for soft water, 4 ozs. for moderately hard water and 6 ozs. for hard water, per 12 gal. of solution.

#### DEVELOPMENT OF IMMERSION CLEANING.

The original farm trials with Immersion Cleaning (Thiel et al., 1955) were made using 5% and 1% solutions of caustic soda. At the farms using the 5% solution, the equipment on removal from the caustic soda solution was first rinsed in a water-rinse tank before being rinsed in hypochlorite-treated water, the water being kept for the whole month. It was found that at the end of the month, the concentrations of the caustic soda solutions had dropped to approximately two-thirds of their original values and the water-rinse in the case of the 5% solution had increased in alkalinity to approximately 0.5% - 1% caustic soda. Bacterial counts were found to be very low in the 5% solution, but higher in the 1% solution and in the water-rinse for the 5% solution. From this it was decided to use a concentration of caustic soda intermediate between the two investigated.





Also in the first trials (Thiel et al., 1955) a plain caustic soda solution was used. It was found that during the monthly cycle a deposit was built up on the liners and that this deposit (which consisted at least in part of calcium soap) could be removed by soaking in an acid solution. Although the milk drawn through liners treated by Immersion Cleaning and the immersion solutions were low in bacterial count, counts on rinses from clusters were surprisingly high, often over 50,000/cluster (The standard of 50,000/cluster was suggested by Clegg & Hoy, 1957 and Clegg et al., 1959, for clusters in fair condition). It was concluded that bacteria must grow in or on the rubber surfaces or in the deposited material on those surfaces.

The incorporation of EDTA into the immersion solution (Thiel et al., 1956) prevented this deposit and produced a better bacteriological condition of the liners. In areas where the water supply was very hard, deposits were formed after the third week of using the solution if only 4 ozs. EDTA/12 gal was used and so for hard water areas, 6 ozs./12 gal was advocated. On some farms a gelatinous material was formed on the rubber, particularly on the parts which were in contact with the teats and this helped to increase the bacterial count. In order to try to reduce the bacterial content of liners, rubber containing bactericides was investigated by Thiel et al. (1956) and Cousins et al. (1957). Thiel et al. in a limited trial found that of the bactericides investigated, only tetramethylthiuram disulphide (TMTD) remained effective in farm use. They also found that rubber containing TMTD was stiffer than normal rubber and was prone to fatigue





splitting, and this was confirmed by Cousins et al., who also found that rubber containing TMTD absorbed more fat. The conclusion from these trials was that rubber containing TMTD did not give better rinse counts than controls and while TMTD rubber was effective in reducing bacterial numbers in the absence of visible milk solids on the rubber, this was not so in the presence of milk solids when such an effect is most needed.

Carreira et al. (1955) compared the milk produced by farms using steam, hypochlorite and Immersion Cleaning for sanitizing the milking equipment and concluded that the milk produced on farms using Immersion Cleaning had a slightly inferior keeping quality to that produced when steam was used, but had a superior keeping quality to the milk produced when hypochlorite was used. The equipment on the farms using steam and hypochlorite was sanitized after washing and brushing in hot detergent solution, whereas, of course, the equipment on the farms using Immersion Cleaning was not brushed daily.



## IMMERSION CLEANING TRIALS IN CANADA

### A. TRIALS WITH BUCKET-MACHINE MILKING EQUIPMENT WITH LONG MILK-TUBES.

The method of Immersion Cleaning was tried on two farms using bucket-machine milking equipment. On Farm I, 2 milking units were used and on Farm II, 3 milking units. Both farms used a refrigerated bulk tank and the milk was carried in pails from the milking barn into the adjoining milk-room where it was emptied into the bulk tank through a strainer. The milk was cooled in the tank to below 40°F and the tank emptied on alternate days.

#### Description of cleaning technique

On both farms all the rubberware was renewed at the beginning of the experiment. The equipment which was not treated by Immersion Cleaning (i.e. buckets, pails, strainers and the bulk tank) was rinsed with water after use and was then brushed in hot detergent solution and, after rinsing with water again, was allowed to drain until just before next milking when it was rinsed in a solution of hypochlorite.

The liners used were deLaval with deep skirts and, before immersion, these were protruded past the ends of the shells in order to allow free access of solution to and from the cavity between the



liners and the shells. The lid gaskets, lid cocks, and any other small items were removed and dropped into the basket. The double vacuum tubes were also placed in the basket along with the long milk tubes. This was not altogether necessary but it was thought that this would help to preserve the rubber. After a while the procedure for rinsing the equipment after removal from the caustic soda solution was changed by the farmer on Farm II. Upon removal from the solution, the equipment was hosed down with water and then the chlorinated rinse was sucked through the assembled machines and the buckets were rinsed out with this solution.

For the first 18 months of the trial the caustic soda solutions were changed monthly by personnel from the Dairy Science Department of the university, and after this, by the farmers themselves. The fresh solutions were made by filling the rinsed-out bins up to the level of the handles (approximately 12 gal) with tap water. On Farm I it was found that if the bin was filled with the first water taken from the tap, when the solution was made there was a red-brown precipitate formed on the top of the solution. This precipitate was considerable and had to be skimmed off. The water on this farm was particularly rich in iron. However, when the tap was allowed to run for a while before putting water into the bin, this precipitate was not formed which indicated that the iron which presumably caused the precipitate probably came from the pipes which conduct the water from the well. The EDTA together with the 3-1/2 lbs commercial caustic soda was added to the water and stirred until all had dissolved. If the solution was not stirred, the caustic soda settled to the bottom of the solution where it formed a



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hard cake which was then difficult to dissolve.

On the same day that the solutions were changed, the clusters were taken apart and everything was brushed in a hot detergent solution. The liners were brushed with the type of brush normally used and the long milk tubes were scraped by pushing a metal rod through them. The rubberware was inspected for damage or wear. All the metal utensils and parts of utensils were examined for milk-stone and whether or not this was visible they were treated by soaking in a hot (approximately 140°F) 4% solution of a proprietary acid milk-stone remover for 30 min, and were brushed intermittently. After this treatment the metal parts were rinsed in the detergent solution. All the metal parts of the equipment were of stainless steel and so neither the caustic soda treatment nor the acid treatment had any noticeable effect on the metal. This was the only time that the clusters were taken apart and brushed in detergent solution.



## I Bacteriological Methods

### Sampling.

For the first six months of the trial (July 6 - Dec. 21, 1959) samples were taken at weekly intervals. After this, samples were taken only at monthly intervals at the same time as the solutions were changed.

The farmers were requested to take three milk samples. These were i) a sample of milk taken from the first bucket or pail the evening previous to the day on which the samples were collected, ii) a sample of the milk taken from the first bucket or pail on the morning of the day when the samples were collected, and iii) a sample of milk taken from the bulk tank. Since the milk was collected from the bulk tank by the dairy every other day, this third sample might be a sample of one day's milk or of two days' milk.

The same day that the milk samples were collected, two clusters from each farm were rinsed. The rinses were taken in the afternoon (i.e. approximately 5 - 6 hr after the equipment had been placed in the immersion solution). The clusters were rinsed by taking them from the solution, allowing them to drain for approximately 2 min and, after replacing the bung in the clawpieces, fitting the cluster assembly into a rack which held the four teat cups in an upright position. The long milk tube was attached and the free end of this was held on a level with the highest part of the liners. Five hundred ml of sterile buffered



quarter-strength Ringer's solution was poured through the cluster, some being poured through each teat cup until the whole cluster was filled with the rinse (The capacity of the cluster assembly is approximately 500 ml). The solution was then drained from the cluster assembly via the long milk tube back into the sterile rinse bottle. This rinse was repeated so that the cluster assembly was rinsed twice with the same solution. Another 500 ml of rinse was run into one of the buckets, the lid and gasket replaced and the whole bucket shaken so that all the surfaces were wetted by the rinse. After allowing to stand for approximately 5 min, the bucket was again shaken and the rinse tipped back into the bottle. If, on the day of sampling, the bulk tank was found empty, swabs were taken from a square foot of the base of the tank, the impeller and the outlet. These tests on the bulk tank and rinses of the buckets were discontinued after it was realized that these articles had not been sanitized but only washed in detergent. They were, as mentioned previously, sanitized with hypochlorite immediately before use and so when the samples were taken they had not received this sanitizing treatment. It would have been possible to rinse these articles with hypochlorite ourselves and then rinse or swab but this was decided against, as it was possible that we might not have done this with the same degree of efficiency as the farmers. Plate counts on the bucket rinses and tank swabs were often very high.

On Farm II the milk samples were refrigerated as soon as convenient after taking. On Farm I, to estimate the effect of limited cooling, the samples were left in the dairy until they were collected.





### Laboratory examination of samples.

The milk samples and rinses, when collected, were placed in a thermally insulated case and taken back to the laboratory where they were left in the refrigerator at about 40°F overnight. They were tested the following morning.

Tests on milk samples. A Standard Plate Count, Methylene Blue Test and a limited Coliform Test were carried out on the samples.

- a) The Standard Plate Count was performed according to the method given in the American Standard Methods for the Examination of Dairy Products (1960).
- b) The Methylene Blue Test, also carried out according to Standard Methods, consisted of adding 1 ml of methylene blue reagent to approximately 10 ml of milk in a sterile screw-capped test tube. This was then inverted to mix the contents and transferred to a water-bath maintained at 37°C. After half an hour the tubes were inverted twice and examined. Subsequently the tubes were examined every half hour and inverted once every hour. When the blue colour of the methylene blue had been partly or completely reduced to colourless as compared with a control tube of milk without methylene blue, the tube was noted as being positive and the number of hours (to the nearest half hour) taken to reach this end-point was recorded. Samples of morning milk were aged for 24 hr at room temperature prior to testing in order to be of an approximately comparable age to the other samples. This was discontinued after 6 months of the trial.





c) The Coliform Test. Only a simple Coliform Test was carried out to give an indication of the coliform content of the milk samples. Tests were carried out on the undiluted milk and also on the first three serial dilutions of the sample (i.e.  $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$ ). One ml of each of these four dilutions was inoculated into separate tubes containing approximately 5 ml of McConkey broth containing a fermentation tube. The tubes were incubated at  $32^{\circ}\text{C}$  for 2 days, and a positive tube regarded as one producing both acid and at least 5% of the Durham tube filled with gas.

Tests on cluster rinses. A standard plate count was made on the rinses from the clusters. Also a standard plate count was made on rinses from buckets and also on swabs from the bulk tank when these samples were taken.

Tests on water samples. On two occasions samples of the water supply were taken and examined by carrying out a standard plate count according to the American Standard Methods for the Examination of Water and Waste-Water (1960) and also a simple coliform test.



### Bacteriological Results

Milk samples. The samples were labelled Bulk, AM and PM, the Bulk sample being the one taken from the bulk tank, AM being the morning milk and PM being the previous evening's milk.

a) Standard plate counts. The results of the standard plate counts are given in Table 1, and the logarithms of these counts in Table 2. In Table 3 the geometric means are calculated for each of the six samples and for each farm, i.e. combining the three samples from each farm. Table 4 shows the frequencies of counts in different classes of log. colony count/ml and these results are plotted in the form of histograms in Figs. 2 and 3. Also in Figs. 2 and 3 are given the percentages of colony counts a) below 200,000/ml, and b) below 50,000/ml.



Table 1. Colony counts on milk samples from two experimental farms over a 2 year period

Date of sample	Standard plate counts x 10 <sup>3</sup> (2 days at 32°C)/ml of milk samples						Mean atmospheric temp.
	Farm I (samples not refrigerated)			Farm II (samples refrigerated)			
	Bulk	AM	PM	Bulk	AM	PM	
6 July (1959)	14 #	12 #	42 #	250 *	28 *	49 *	59
14 July	19 #	53 #	330 #	43 *	53 *	40 *	67
22 July	11 #	1 #	110 #	15 *	250 *	3,000 *	70
28 July	20 #	1 #	9 #	10 *	4 *	37 *	57
3 Aug.	31	3	1,100	7	3	1	62
10 Aug.	14	32	94	6	1	2	53
18 Aug.	40	190	8.2	13	4.8	9.8	53
26 Aug.	37	41	3	6	5	3	55
9 Sept.	25	17	16	6	12	6	49
15 Sept.	210	24	38	5.5	4.5	4	49
21 Sept.	34	22	7.8	8.6	3.5	4.8	53
28 Sept.	12	55	4.5	8.4	35	290	37
6 Oct.	30	15	11	5.8	22	1.1	30
14 Oct.	33	5.6	8.8	16	4	5.9	46
22 Oct.	14	19	13	8.4	11	1.4	43
28 Oct.	28	18	8.8	11	4.9	22	37
3 Nov.	59	22	34	35	30	7.5	19
10 Nov.	61	62	26	12	15	13	17
17 Nov.	26	41	26	24	35	16	24
24 Nov.	19	19	21	11	17	3.2	33
30 Nov.	29	29	31	19	14	7.8	31
7 Dec.	26	23	28	18	7.3	9.9	29
15 Dec.	18	12	15	33	17	15	32
21 Dec.	34	16	34	23	14	6.9	20
11 Jan. (1960)	20	8.8	26	32	32	41	13
1 Feb.	30	20	54	30	50	9.5	26
29 Feb.	26	6.8	18	39	6.2	46	4
28 Mar.	18	5.6	13	24	75	600	20
3 May	19	11	8.3	15	2.1	4.6	39
1 June	19	61	510	13	11	9.9	52
4 July	160	4,400	17,000	26	16	9.1	63
9 Aug.	-	-	-	15	8.2	6.8	69
12 Sept.	4,300 #	22,000 #	54,000 #	17	3.4	4.1	62
21 Oct.	48	64	130	15	7.6	1.5	39
7 Nov.	45	9.8	12	18	28	1.4	27
12 Dec.	25	900	380	37	6.5	6.8	28
10 Jan. (1961)	20	15	10	15	7.1	4.9	47
6 Feb.	7.8	1.5	3	20	7.1	7.9	24
6 Mar.	20	11	7.6	10	12	12	9
12 Apr.	31	37	61	220 *	43 *	170 *	41
8 May	26	26	40	17	18	5.5	38
15 June	14	3.4	31	1,200 *	5,800 *	20,000 *	

# samples refrigerated.

\* samples not refrigerated.

≠ samples in sun in milk-house

Mean atmospheric temp.

= mean  $(^{\circ}\text{F})$  from 24 hourly readings.

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Table 2. Logarithmic transformation of colony counts from milk samples in Table 1

Date of sample	Logarithms of the standard plate counts x 10 <sup>3</sup> / ml of milk samples						Mean atm- ospheric temp.
	Farm I (samples not refrigerated)			Farm II (samples refrigerated)			
	Bulk	AM	PM	Bulk	AM	PM	
6 July (1959)	1.1461	1.0792	1.6232	2.3979	1.4472	1.6902	59
14 July	1.2788	1.7243	2.5185	1.6335	1.7243	1.6021	67
22 July	1.0414	0.0000	2.0414	1.1761	2.3070	3.4771	70
28 July	1.3010	0.0000	0.9542	1.0000	0.6021	1.5682	57
10 Aug.	1.1461	1.5051	1.9731	0.7782	0.0000	0.3010	53
18 Aug.	1.6021	2.2788	0.9138	1.1139	0.6812	0.9912	53
26 Aug.	1.5682	1.6128	2.4771	0.7782	0.6990	0.4771	55
9 Sept.	1.3979	1.2304	1.2041	0.7782	1.0792	0.7782	49
15 Sept.	2.3222	1.3802	1.5798	0.7404	0.6532	0.6021	49
21 Sept.	1.5315	1.3424	0.8921	0.9345	0.5441	0.6812	53
28 Sept.	1.0792	1.7404	0.6532	0.9243	1.5441	2.4624	37
6 Oct.	1.4771	1.1761	1.0414	0.7634	1.3424	0.0414	30
14 Oct.	1.5185	0.7482	0.9445	1.2041	0.6021	0.7709	46
22 Oct.	1.1461	1.2788	1.1139	0.9243	1.0414	0.1461	43
28 Oct.	1.4472	1.2553	0.9445	1.0414	0.6902	1.3424	37
3 Nov.	1.7709	1.3424	1.5315	1.5441	1.4771	0.8751	19
10 Nov.	1.7853	1.7924	1.4150	1.0792	1.1761	1.1139	17
17 Nov.	1.4150	1.6128	1.4150	1.3802	1.5441	1.2041	24
24 Nov.	1.2788	1.2788	1.3222	1.0414	1.2304	0.5051	33
30 Nov.	1.4624	1.4624	1.4014	1.2788	1.1461	0.8921	31
7 Dec.	1.4150	1.3617	1.4472	1.2553	0.8633	0.9956	29
15 Dec.	1.2553	1.0792	1.1761	1.5185	1.2304	1.1761	32
21 Dec.	1.5315	1.2041	1.5315	1.3617	1.1461	0.8388	20
11 Jan. (1960)	1.3010	0.9445	1.4150	1.5051	1.5051	1.6128	13
1 Feb.	1.4771	1.3010	1.7324	1.4771	1.6990	0.9777	26
29 Feb.	1.4150	0.8325	1.2553	1.5911	0.7924	1.6628	-4
28 Mar.	1.2553	0.7482	1.1139	1.3802	1.8751	2.7782	20
3 May	1.2788	1.0414	0.9191	1.1761	0.3222	0.6628	39
1 June	1.2788	1.7853	2.7076	1.1139	1.0414	0.9956	52
4 July	2.2041	3.6434	4.2304	1.4150	1.2041	0.9590	63
9 Aug.	-	-	-	1.1761	0.9138	0.8325	69
12 Sept.	3.6335	4.3424	4.7324	1.2304	0.5315	0.6128	62
21 Oct.	1.6812	1.8062	2.1139	1.1761	0.8808	0.1761	39
7 Nov.	1.6532	0.9912	1.0792	1.2553	1.4472	0.1461	27
12 Dec.	1.3979	2.0542	2.5798	1.5682	0.8129	0.8325	28
10 Jan. (1961)	1.3010	1.1761	1.0000	1.1761	0.8513	0.6902	47
6 Feb.	0.8921	0.1761	0.4771	1.3010	0.8513	0.8976	24
6 Mar.	1.3010	1.0414	0.8808	1.0000	1.0792	1.0792	9
12 Apr.	1.4914	1.5682	1.7853	2.3424	1.6335	2.2304	41
8 May	1.4150	1.4150	1.6021	1.2304	1.2553	0.7404	38
15 June	1.1461	0.5315	1.4914	3.0792	3.7634	4.3010	

Mean atmospheric temp.  
= mean ( $^{\circ}\text{F}$ ) from 24  
hourly readings.



Table 3. The geometric means for each type of milk sample and for each farm

	Mean colony count $\times 10^3$ /ml from:					
	Farm I			Farm II		
	Bulk	AM	PM	Bulk	AM	PM
Sum of logs.	60.5315	56.2616	64.2718	53.6864	47.7986	46.7201
No. of samples	41	41	41	42	42	42
Mean	1.4764	1.3722	1.5676	1.2782	1.1381	1.1124
Antilog. (i.e. Geom. mean)	<u>29.95</u>	<u>23.56</u>	<u>36.95</u>	<u>18.98</u>	<u>13.74</u>	<u>12.95</u>
Sum of logs. for each farm	181.0648			148.0051		
No. of samples	123			126		
Mean	1.4723			1.1746		
Antilog. (i.e. Geom. mean)	<u>29.67</u>			<u>14.95</u>		



Table 4. Frequency distribution of colony counts of milk samples from two experimental farms

Log. colony count $\times 10^3$ /ml milk	Number of individual samples from:							
	Farm I				Farm II			
	Bulk	AM	PM	Sum	Bulk	AM	PM	Sum
0.0 - 0.5	0	4	2	6	0	3	7	10
0.5 - 1.0	1	6	9	16	11	15	20	46
1.0 - 1.5	28	17	15	60	22	15	5	42
1.5 - 2.0	9	10	7	26	6	7	5	18
2.0 - 2.5	2	1	2	5	2	1	2	5
2.5 - 3.0	0	1	3	4	0	0	1	1
3.0 - 3.5	0	0	1	1	1	0	1	2
3.5 - 4.0	1	1	0	2	0	1	0	1
4.0 - 4.5	0	1	1	2	0	0	1	1
4.5 - 5.0	0	0	1	1	0	0	0	0

Data pertaining to Fig. 2 and 3.

1. The first part of the document is a list of names and addresses of the members of the committee.

List of Members						Total
Name	Address	City	State	Country	Occupation	
Mr. A. B. C.	123 Main St.	New York	NY	USA	Engineer	100
Mr. D. E. F.	456 Elm St.	Los Angeles	CA	USA	Teacher	50
Mr. G. H. I.	789 Oak St.	Chicago	IL	USA	Doctor	75
Mr. J. K. L.	101 Pine St.	San Francisco	CA	USA	Lawyer	120
Mr. M. N. O.	202 Cedar St.	London	UK	UK	Artist	30
Mr. P. Q. R.	303 Birch St.	Paris	FR	FR	Writer	40
Mr. S. T. U.	404 Maple St.	Berlin	DE	DE	Musician	20
Mr. V. W. X.	505 Spruce St.	Moscow	RU	RU	Scientist	60
Mr. Y. Z. A.	606 Willow St.	Tokyo	JP	JP	Businessman	80
Mr. B. C. D.	707 Ash St.	Sydney	AU	AU	Journalist	10
Mr. E. F. G.	808 Hickory St.	Melbourne	AU	AU	Actor	15
Mr. H. I. J.	909 Sycamore St.	Auckland	NZ	NZ	Politician	5
Mr. K. L. M.	1010 Dogwood St.	Wellington	NZ	NZ	Engineer	10
Mr. N. O. P.	1111 Redwood St.	Christchurch	NZ	NZ	Teacher	5
Mr. Q. R. S.	1212 Juniper St.	Dunedin	NZ	NZ	Doctor	5
Mr. T. U. V.	1313 Cypress St.	Hamilton	NZ	NZ	Lawyer	5
Mr. W. X. Y.	1414 Fir St.	Palmerston North	NZ	NZ	Businessman	5
Mr. Z. A. B.	1515 Pine St.	Wellington	NZ	NZ	Engineer	5
Mr. C. D. E.	1616 Oak St.	Christchurch	NZ	NZ	Teacher	5
Mr. F. G. H.	1717 Elm St.	Dunedin	NZ	NZ	Doctor	5
Mr. I. J. K.	1818 Maple St.	Hamilton	NZ	NZ	Lawyer	5
Mr. L. M. N.	1919 Birch St.	Palmerston North	NZ	NZ	Businessman	5
Mr. O. P. Q.	2020 Spruce St.	Wellington	NZ	NZ	Engineer	5
Mr. R. S. T.	2121 Willow St.	Christchurch	NZ	NZ	Teacher	5
Mr. U. V. W.	2222 Ash St.	Dunedin	NZ	NZ	Doctor	5
Mr. X. Y. Z.	2323 Hickory St.	Hamilton	NZ	NZ	Lawyer	5
Mr. A. B. C.	2424 Sycamore St.	Palmerston North	NZ	NZ	Businessman	5
Mr. D. E. F.	2525 Dogwood St.	Wellington	NZ	NZ	Engineer	5
Mr. G. H. I.	2626 Redwood St.	Christchurch	NZ	NZ	Teacher	5
Mr. J. K. L.	2727 Juniper St.	Dunedin	NZ	NZ	Doctor	5
Mr. M. N. O.	2828 Cypress St.	Hamilton	NZ	NZ	Lawyer	5
Mr. P. Q. R.	2929 Fir St.	Palmerston North	NZ	NZ	Businessman	5
Mr. S. T. U.	3030 Pine St.	Wellington	NZ	NZ	Engineer	5
Mr. V. W. X.	3131 Oak St.	Christchurch	NZ	NZ	Teacher	5
Mr. Y. Z. A.	3232 Elm St.	Dunedin	NZ	NZ	Doctor	5
Mr. B. C. D.	3333 Maple St.	Hamilton	NZ	NZ	Lawyer	5
Mr. E. F. G.	3434 Birch St.	Palmerston North	NZ	NZ	Businessman	5
Mr. H. I. J.	3535 Spruce St.	Wellington	NZ	NZ	Engineer	5
Mr. K. L. M.	3636 Willow St.	Christchurch	NZ	NZ	Teacher	5
Mr. N. O. P.	3737 Ash St.	Dunedin	NZ	NZ	Doctor	5
Mr. Q. R. S.	3838 Hickory St.	Hamilton	NZ	NZ	Lawyer	5
Mr. T. U. V.	3939 Sycamore St.	Palmerston North	NZ	NZ	Businessman	5
Mr. W. X. Y.	4040 Dogwood St.	Wellington	NZ	NZ	Engineer	5
Mr. Z. A. B.	4141 Redwood St.	Christchurch	NZ	NZ	Teacher	5
Mr. C. D. E.	4242 Juniper St.	Dunedin	NZ	NZ	Doctor	5
Mr. F. G. H.	4343 Cypress St.	Hamilton	NZ	NZ	Lawyer	5
Mr. I. J. K.	4444 Fir St.	Palmerston North	NZ	NZ	Businessman	5
Mr. L. M. N.	4545 Pine St.	Wellington	NZ	NZ	Engineer	5
Mr. O. P. Q.	4646 Oak St.	Christchurch	NZ	NZ	Teacher	5
Mr. R. S. T.	4747 Elm St.	Dunedin	NZ	NZ	Doctor	5
Mr. U. V. W.	4848 Maple St.	Hamilton	NZ	NZ	Lawyer	5
Mr. X. Y. Z.	4949 Birch St.	Palmerston North	NZ	NZ	Businessman	5
Mr. A. B. C.	5050 Spruce St.	Wellington	NZ	NZ	Engineer	5
Mr. D. E. F.	5151 Willow St.	Christchurch	NZ	NZ	Teacher	5
Mr. G. H. I.	5252 Ash St.	Dunedin	NZ	NZ	Doctor	5
Mr. J. K. L.	5353 Hickory St.	Hamilton	NZ	NZ	Lawyer	5
Mr. M. N. O.	5454 Sycamore St.	Palmerston North	NZ	NZ	Businessman	5
Mr. P. Q. R.	5555 Dogwood St.	Wellington	NZ	NZ	Engineer	5
Mr. S. T. U.	5656 Redwood St.	Christchurch	NZ	NZ	Teacher	5
Mr. V. W. X.	5757 Juniper St.	Dunedin	NZ	NZ	Doctor	5
Mr. Y. Z. A.	5858 Cypress St.	Hamilton	NZ	NZ	Lawyer	5
Mr. B. C. D.	5959 Fir St.	Palmerston North	NZ	NZ	Businessman	5
Mr. E. F. G.	6060 Pine St.	Wellington	NZ	NZ	Engineer	5
Mr. H. I. J.	6161 Oak St.	Christchurch	NZ	NZ	Teacher	5
Mr. K. L. M.	6262 Elm St.	Dunedin	NZ	NZ	Doctor	5
Mr. N. O. P.	6363 Maple St.	Hamilton	NZ	NZ	Lawyer	5
Mr. Q. R. S.	6464 Birch St.	Palmerston North	NZ	NZ	Businessman	5
Mr. T. U. V.	6565 Spruce St.	Wellington	NZ	NZ	Engineer	5
Mr. W. X. Y.	6666 Willow St.	Christchurch	NZ	NZ	Teacher	5
Mr. Z. A. B.	6767 Ash St.	Dunedin	NZ	NZ	Doctor	5
Mr. C. D. E.	6868 Hickory St.	Hamilton	NZ	NZ	Lawyer	5
Mr. F. G. H.	6969 Sycamore St.	Palmerston North	NZ	NZ	Businessman	5
Mr. I. J. K.	7070 Dogwood St.	Wellington	NZ	NZ	Engineer	5
Mr. L. M. N.	7171 Redwood St.	Christchurch	NZ	NZ	Teacher	5
Mr. O. P. Q.	7272 Juniper St.	Dunedin	NZ	NZ	Doctor	5
Mr. R. S. T.	7373 Cypress St.	Hamilton	NZ	NZ	Lawyer	5
Mr. U. V. W.	7474 Fir St.	Palmerston North	NZ	NZ	Businessman	5
Mr. X. Y. Z.	7575 Pine St.	Wellington	NZ	NZ	Engineer	5
Mr. A. B. C.	7676 Oak St.	Christchurch	NZ	NZ	Teacher	5
Mr. D. E. F.	7777 Elm St.	Dunedin	NZ	NZ	Doctor	5
Mr. G. H. I.	7878 Maple St.	Hamilton	NZ	NZ	Lawyer	5
Mr. J. K. L.	7979 Birch St.	Palmerston North	NZ	NZ	Businessman	5
Mr. M. N. O.	8080 Spruce St.	Wellington	NZ	NZ	Engineer	5
Mr. P. Q. R.	8181 Willow St.	Christchurch	NZ	NZ	Teacher	5
Mr. S. T. U.	8282 Ash St.	Dunedin	NZ	NZ	Doctor	5
Mr. V. W. X.	8383 Hickory St.	Hamilton	NZ	NZ	Lawyer	5
Mr. Y. Z. A.	8484 Sycamore St.	Palmerston North	NZ	NZ	Businessman	5
Mr. B. C. D.	8585 Dogwood St.	Wellington	NZ	NZ	Engineer	5
Mr. E. F. G.	8686 Redwood St.	Christchurch	NZ	NZ	Teacher	5
Mr. H. I. J.	8787 Juniper St.	Dunedin	NZ	NZ	Doctor	5
Mr. K. L. M.	8888 Cypress St.	Hamilton	NZ	NZ	Lawyer	5
Mr. N. O. P.	8989 Fir St.	Palmerston North	NZ	NZ	Businessman	5
Mr. Q. R. S.	9090 Pine St.	Wellington	NZ	NZ	Engineer	5
Mr. T. U. V.	9191 Oak St.	Christchurch	NZ	NZ	Teacher	5
Mr. W. X. Y.	9292 Elm St.	Dunedin	NZ	NZ	Doctor	5
Mr. Z. A. B.	9393 Maple St.	Hamilton	NZ	NZ	Lawyer	5
Mr. C. D. E.	9494 Birch St.	Palmerston North	NZ	NZ	Businessman	5
Mr. F. G. H.	9595 Spruce St.	Wellington	NZ	NZ	Engineer	5
Mr. I. J. K.	9696 Willow St.	Christchurch	NZ	NZ	Teacher	5
Mr. L. M. N.	9797 Ash St.	Dunedin	NZ	NZ	Doctor	5
Mr. O. P. Q.	9898 Hickory St.	Hamilton	NZ	NZ	Lawyer	5
Mr. R. S. T.	9999 Sycamore St.	Palmerston North	NZ	NZ	Businessman	5

2. The second part of the document is a list of names and addresses of the members of the committee.

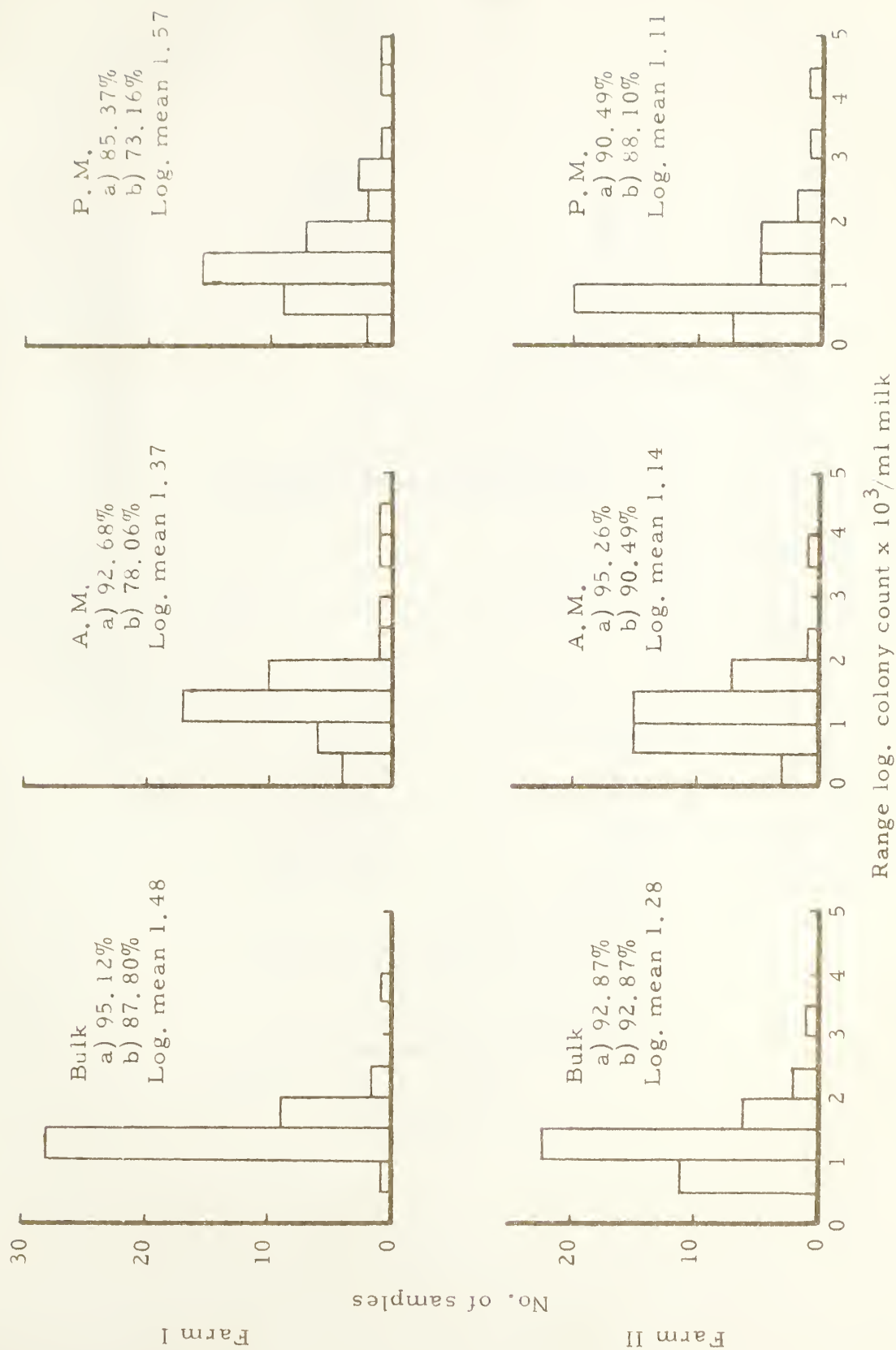


Fig. 2. Frequency distribution of colony counts of milks from two experimental farms. a) percentage of samples below 200,000/ml. b) percentage of samples below 50,000 ml





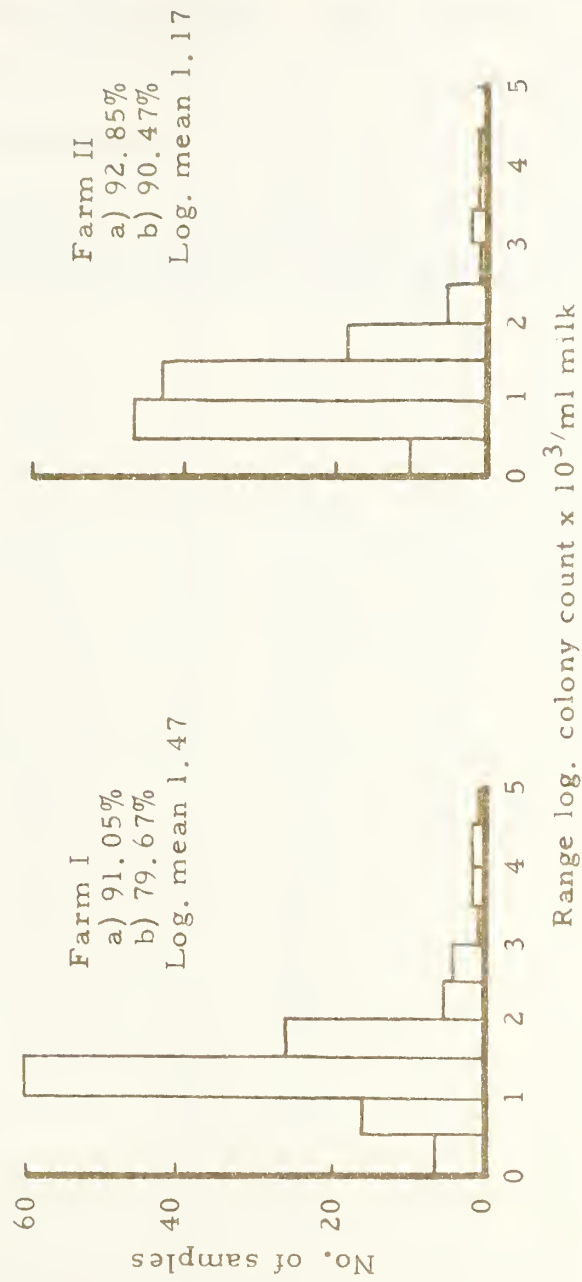


Fig. 3. Frequency distribution of colony counts of all samples of milk from each of the two experimental farms.  
a) percentage of samples below 200,000/ml, b) percentage of samples below 50,000/ml



b) Methylene blue test. Table 5 shows the results of the methylene blue tests on the milk samples. The percentages of samples having a reduction time of 7 or more hr and the percentages of samples having a reduction time of 5-1/2 hr or more (the standard for Grade A raw milk to be pasteurized given in American Standard Methods for the Examination of Dairy Products, 1960) are given in Table 6.

and the other side of the river, the water is very shallow (

about 1 foot deep) and the water is very muddy (the water is very

dirty, and the water is very muddy, and the water is very muddy)

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Table 5. Methylene Blue Reduction Tests on milk samples from two experimental farms

Date of sample	Times to reduction of methylene blue (hr)						Mean atmos- pheric temp.
	Farm I			Farm II			
	(samples not refrigerated)			(samples refrigerated)			
	Bulk	AM	PM	Bulk	AM	PM	
6 July (1959)	-	-	-	-	-	-	
14 July	7+ #	7+ #	7+ #	7+ *	7+ *	7+ *	67
22 July	7+ #	6 #	4 #	6 *	7+ *	4 *	70
28 July	7+ #	7+ #	7+ #	7+ *	5.5 *	4 *	57
3 Aug.	7+	7+	5	7+	6.5	7+	62
10 Aug.	7+	7+	7+	7+	7+	7+	53
18 Aug.	7+	7+	6	7+	7+	7+	53
26 Aug.	-	-	-	-	-	-	
9 Sept.	7+	7+	7+	7+	7+	7+	49
15 Sept.	7+	7+	7+	7+	7+	7+	49
21 Sept.	7+	7+	7+	7+	7+	7+	53
28 Sept.	7+	7+	7+	7+	7	6.5	37
6 Oct.	7+	7+	7+	7+	7+	6	30
14 Oct.	7+	7+	7+	7+	7+	7+	46
22 Oct.	7+	7+	7+	7+	7+	7+	43
28 Oct.	7+	7+	7+	7+	7+	7+	37
3 Nov.	7+	7+	7+	7	7+	7+	19
10 Nov.	7+	7+	7+	7+	2	7+	17
17 Nov.	7+	3	7+	7+	3.5	7+	24
24 Nov.	7+	5.5	7+	7+	5.5	7+	33
30 Nov.	7+	1.5	7+	7+	1.5	7+	31
7 Dec.	7+	2	7+	7+	4.5	7+	29
15 Dec.	7+	7+	7+	7+	7+	7+	32
21 Dec.	7+	7+	7+	7+	7+	7+	20
11 Jan. (1960)	7+	7+	7+	7+	7+	7	13
1 Feb.	7+	7+	7+	7+	7+	7+	26
29 Feb.	7+	7+	7+	7+	7+	7	-4
28 Mar.	7+	7+	7+	7+	7+	7	20
3 May	7+	7+	7+	7+	7+	7+	39
1 June	7	7+	4.5	7+	7+	7+	52
4 July	4.5	2.5	1	7	7	5	63
9 Aug.	-	-	-	7+	7+	7	69
12 Sept.	3.5	2	1	7+	7+	7	62
21 Oct.	7+	7+	4.5	7+	7+	7+	39
7 Nov.	7+	7+	7+	7+	7+	7+	27
12 Dec.	7+	7+	7+	7+	7+	7+	28
10 Jan. (1961)	7+	7+	7+	7+	7+	7+	47
6 Feb.	7+	7+	7+	7+	7+	?	24
6 Mar.	7+	7+	7+	7+	7+	7+	9
12 Apr.	7+	7+	7+	4 *	6 *	4 *	41
8 May	7+	7+	7+	7+	7+	7+	38
15 June	7+	7+	7+	5 *	4 *	1 *	

# samples refrigerated.

\* samples not refrigerated.





Table 6. A comparison of different standards of the methylene blue test on the two experimental farms

Type of sample	Percentage of samples having reduction times of	
	7 or more hr	5-1/2 or more hr
<u>Farm I</u>		
Bulk	92.3	94.9
AM	52.0	87.2
PM	82.0	84.6
<u>Farm II</u>		
Bulk	87.5	95.0
AM	72.5	87.5
PM	69.2	87.2



c) Coliform test. The results of the coliform test are tabulated in Table 7. The percentages in different groups are given in Table 8.

(2)

2000

2000

Table 7. Coliform contents of milk samples from two experimental farms

Date of sample	Coliform content of samples						Mean atmos- pheric temp.
	Farm I (samples not refrigerated)			Farm II (samples refrigerated)			
	Bulk	AM	PM	Bulk	AM	PM	
6 July (1959)	4	0	0	3	0	0	59
14 July	4	3	4	3	1	4	67
22 July	2	3	4	3	2	4	70
28 July	3	0	2	2	0	0	57
3 Aug.	0	0	4	2	1	0	62
10 Aug.	1	4	4	2	1	3	53
18 Aug.	1	2	0	4	1	0	53
26 Aug.	3	1	0	2	0	1	55
9 Sept.	3	0	1	2	0	0	49
15 Sept.	4	4	3	2	0	0	49
22 Sept.	4	1	0	3	0	0	53
28 Sept.	1	0	0	1	1	0	37
6 Oct.	4	0	0	2	3	4	30
14 Oct.	1	1	0	2	0	0	46
22 Oct.	1	2	2	0	2	1	43
28 Oct.	1	0	0	4	1	0	37
3 Nov.	3	0	1	3	1	3	19
10 Nov.	2	1	0	4	2	2	17
17 Nov.	0	0	0	2	1	0	24
24 Nov.	2	0	1	2	1	0	33
30 Nov.	1	0	0	2	1	1	31
7 Dec.	0	0	0	4	0	1	29
15 Dec.	1	0	0	4	2	1	32
21 Dec.	0	0	0	2	1	1	20
11 Jan. (1960)	3	1	0	2	1	4	13
1 Feb.	0	1	0	2	2	1	26
29 Feb.	1	0	0	1	1	2	-4
28 Mar.	0	0	0	2	2	2	20
3 May	2	0	0	3	0	2	39
1 June	1	0	2	2	0	1	52
4 July	4	4	4	2	2	1	63
9 Aug.	-	-	-	1	1	0	69
12 Sept.	4	4	4	2	1	3	62
21 Oct.	3	4	3	2	0	0	39
7 Nov.	3	1	0	1	0	0	27
12 Dec.	2	2	1	1	1	2	28
10 Jan. (1961)	2	0	0	3	2	1	47
6 Feb.	0	0	0	1	1	1	24
6 Mar.	2	0	2	0	1	0	9
12 Apr.	2	0	0	4	2	2	41
8 May	1	0	0	1	0	0	38
15 June	1	0	0	4	4	4	
	77	39	42	94	43	52	

0, coliforms absent in 1 ml; 1, coliforms absent in 0.1 ml;  
 2, coliforms absent in 0.01 ml; 3, coliforms absent in 0.001 ml;  
 4, coliforms present in 0.0001 ml.

Mean atmospheric temp. = mean (°F) from 24 hourly readings.



Table 8. Milk samples from two experimental farms grouped into classes of coliform content

Type of sample	Percentage of milk samples with coliforms				
	absent in (ml)	present in (ml)			
	1	1	0.1	0.01	0.001
<u>Farm I</u>					
Bulk	17.07	29.27	19.51	17.07	17.07
AM	58.54	17.07	7.32	4.88	12.20
PM	60.98	9.76	9.76	4.88	14.63
Total	45.53	18.70	12.20	8.94	14.63
<u>Farm II</u>					
Bulk	4.76	16.67	45.24	16.67	16.67
AM	30.95	42.86	21.43	2.38	2.38
PM	40.48	26.19	14.28	7.14	11.90
Total	25.40	28.57	26.98	8.73	10.32





Cluster rinses. The rinses of clusters on Farm I were labelled A and B and those on Farm II were labelled C and D. The results of the standard plate counts on rinses of clusters are given in Table 9. The logarithms of these counts are presented in Table 10 and in Table 11 are calculated the geometric means for each cluster, for each farm and for the four clusters taken together. The frequencies with which the logarithms of the rinse counts fall into different classes are given in Table 12 and these data are plotted in the form of histograms in Figs. 4 and 5. Also in Figs. 4 and 5 are given the percentages of counts a) below 200,000/cluster, and b) below 50,000/cluster.



Table 9. Colony counts on rinses of clusters from two experimental farms over a 2 year period

Date of sample	Standard plate counts $\times 10^3$ (2 days at 32°C) /cluster				Mean atmospheric temp.
	Farm I		Farm II		
	Cluster A	Cluster B	Cluster C	Cluster D	
6 July (1959)	1	1	24	140	59
14 July	15	15	220	25	67
22 July	0.25	19	400	2.5	70
28 July	3	0.25	23,000	1	57
3 Aug.	0.5	13	28	0.25	62
10 Aug.	1.5	11	1.5	0.5	53
18 Aug.	200	3	1,700	1,100	53
26 Aug.	88	20	32	0.25	55
9 Sept.	150	10	10	10	49
15 Sept.	16	28	7	12	49
21 Sept.	29	23	14	880	53
28 Sept.	6.5	20	0.5	13	37
6 Oct.	26	72	5.5	12	30
14 Oct.	91	29	14	9	46
22 Oct.	18	11	13	6.5	43
28 Oct.	20	28	10	28	37
3 Nov.	27	20	4.5	23	19
10 Nov.	72	29	3	20	17
17 Nov.	52	31	82	97	24
24 Nov.	120	44	84	22	33
30 Nov.	79	47	0.25	340	31
7 Dec.	32	25	61	43	29
15 Dec.	210	180	42	14	32
21 Dec.	36	130	8.5	1	20
11 Jan. (1960)	43	21	24	2.5	13
1 Feb.	69	89	11	230	26
29 Feb.	66	67	5	78	-4
28 Mar.	140	99	110	77	20
3 May	50	22	360	18	39
1 June	25	30	12	28	52
4 July	12	91	1	40	63
9 Aug.	10	8.5	4.5	58	69
12 Sept.	52	200	560	180	62
21 Oct.	38	24	9.5	8	39
7 Nov.	90	98	20	20	27
12 Dec.	-	-	36	60	28
10 Jan. (1961)	320	220	98	42	47
6 Feb.	26	42	32	2.5	24
6 Mar.	52	57	14	8.5	9
12 Apr.	42	14	230	46	41
8 May	0.25	45	18	18	38
15 June	8.5	6	4	5	

Mean atmospheric temp. = mean (°F) from 24 hourly readings.



Table 10. Logarithmic transformations of colony counts from cluster rinses in Table 9

Date of sample	Logarithms of the standard plate counts x 10 <sup>3</sup> /cluster				Mean atmosph- eric temp.
	Farm I		Farm II		
	Cluster		Cluster		
	A	B	C	D	
6 July (1959)	0	0	1.3802	2.1461	59
11 July	1.1761	1.1761	2.3424	1.3979	67
22 July	1.3979	1.2788	2.6021	0.3979	70
28 July	0.4771	1.3979	4.3617	0	57
3 Aug.	1.6990	1.1139	1.4472	1.3979	62
10 Aug.	0.1761	1.0414	0.1761	1.6990	53
18 Aug.	2.3010	0.4771	3.2304	3.0414	53
26 Aug.	1.9445	1.3010	1.5051	1.3979	55
9 Sept.	2.1761	1.0000	1.0000	1.0000	49
15 Sept.	1.2041	1.4472	0.8451	1.0792	49
21 Sept.	1.4624	1.3617	1.1461	2.9445	53
28 Sept.	0.8129	1.3010	1.6990	1.1139	37
6 Oct.	1.4150	1.8573	0.7404	1.0792	30
14 Oct.	1.9590	1.4624	1.1461	0.9542	46
22 Oct.	1.2553	1.0414	1.1139	0.8129	43
28 Oct.	1.3010	1.4472	1.0000	1.4472	37
3 Nov.	1.4314	1.3010	0.6532	1.3617	19
10 Nov.	1.8573	1.4624	0.4771	1.3010	17
17 Nov.	1.7160	1.4914	1.9138	1.9868	24
24 Nov.	2.0792	1.6435	1.9243	1.3424	33
30 Nov.	1.8976	1.6721	1.3979	2.5315	31
7 Dec.	1.5051	1.3979	1.7853	1.6335	29
15 Dec.	2.3222	2.2553	1.6232	1.1461	32
21 Dec.	1.5563	2.1139	0.9294	0	20
11 Jan. (1960)	1.6335	1.3222	1.3802	0.3979	13
1 Feb.	1.8388	1.9494	1.0414	2.3617	26
29 Feb.	1.8195	1.8261	0.6990	1.8921	-4
28 Mar.	2.1461	1.9956	2.0414	1.8865	20
3 May	1.6990	1.3424	2.5563	1.2553	39
1 June	1.3979	1.4771	1.0792	1.4472	52
4 July	1.0792	1.9590	0	1.6021	63
9 Aug.	1.0000	0.9294	0.6532	1.7634	69
12 Sept.	1.7160	2.3010	2.7482	2.2553	62
21 Oct.	1.5798	1.3802	0.9777	0.9031	39
7 Nov.	1.9542	1.9912	1.3010	1.3010	27
12 Dec.	-	-	1.5563	1.7782	28
10 Jan. (1961)	2.5052	2.3424	1.9912	1.6232	47
6 Feb.	1.4150	1.6232	1.5052	0.3979	24
6 Mar.	1.7160	1.7559	1.1461	0.9294	9
12 Apr.	1.6232	1.1461	2.3617	1.6628	41
8 May	1.3979	1.6532	1.2553	1.2553	38
15 June	0.9294	0.7782	0.6021	0.6990	

Mean atmospheric temp. = mean ( $^{\circ}\text{F}$ ) from 24 hourly readings.





Table 11. The geometric means of rinse results for each cluster, each farm and the two farms taken together

	Mean colony count x 10 <sup>3</sup> /cluster			
	Farm I Cluster		Farm II Cluster	
	A	B	C	D
Sum of logs.	56.5733	57.8145	57.3355	52.6236
No. of samples	41	41	42	42
Mean	1.3798	1.4101	1.3651	1.2529
Antilog. (i.e. Geom. mean)	<u>23.98</u>	<u>25.71</u>	<u>23.18</u>	<u>17.90</u>
Sum of logs. for each farm	114.3878		109.9591	
No. of samples	82		84	
Mean	1.3950		1.3090	
Antilog. (i.e. Geom. mean)	<u>24.83</u>		<u>20.37</u>	
Sum of logs. for all clusters	224.3469			
No. of samples	166			
Mean	1.3515			
Antilog. (i.e. Geom. mean)	22.47			



Table 12. Frequency distribution of colony counts of cluster rinses from two experimental farms

Log. colony count x 10 <sup>3</sup> /cluster	Number of individual samples from						Total all clusters
	Farm I			Farm II			
	Cluster A	B	Sum	Cluster C	D	Sum	
1.0 - 1.5	2	1	3	1	2	3	6
1.5 - 2.0	2	1	3	2	3	5	8
2.0 - 2.5	2	1	3	2	3	5	8
2.5 - 3.0	3	3	6	10	6	16	22
3.0 - 3.5	10	20	30	11	13	24	54
3.5 - 4.0	16	11	27	8	9	17	44
4.0 - 4.5	5	4	9	3	3	6	15
4.5 - 5.0	1	0	1	3	2	5	6
5.0 - 5.5	0	0	0	1	1	2	2
5.5 - 6.0	0	0	0	0	0	0	0
6.0 - 6.5	0	0	0	1	0	1	1

Data pertaining to Figs. 4 and 5.



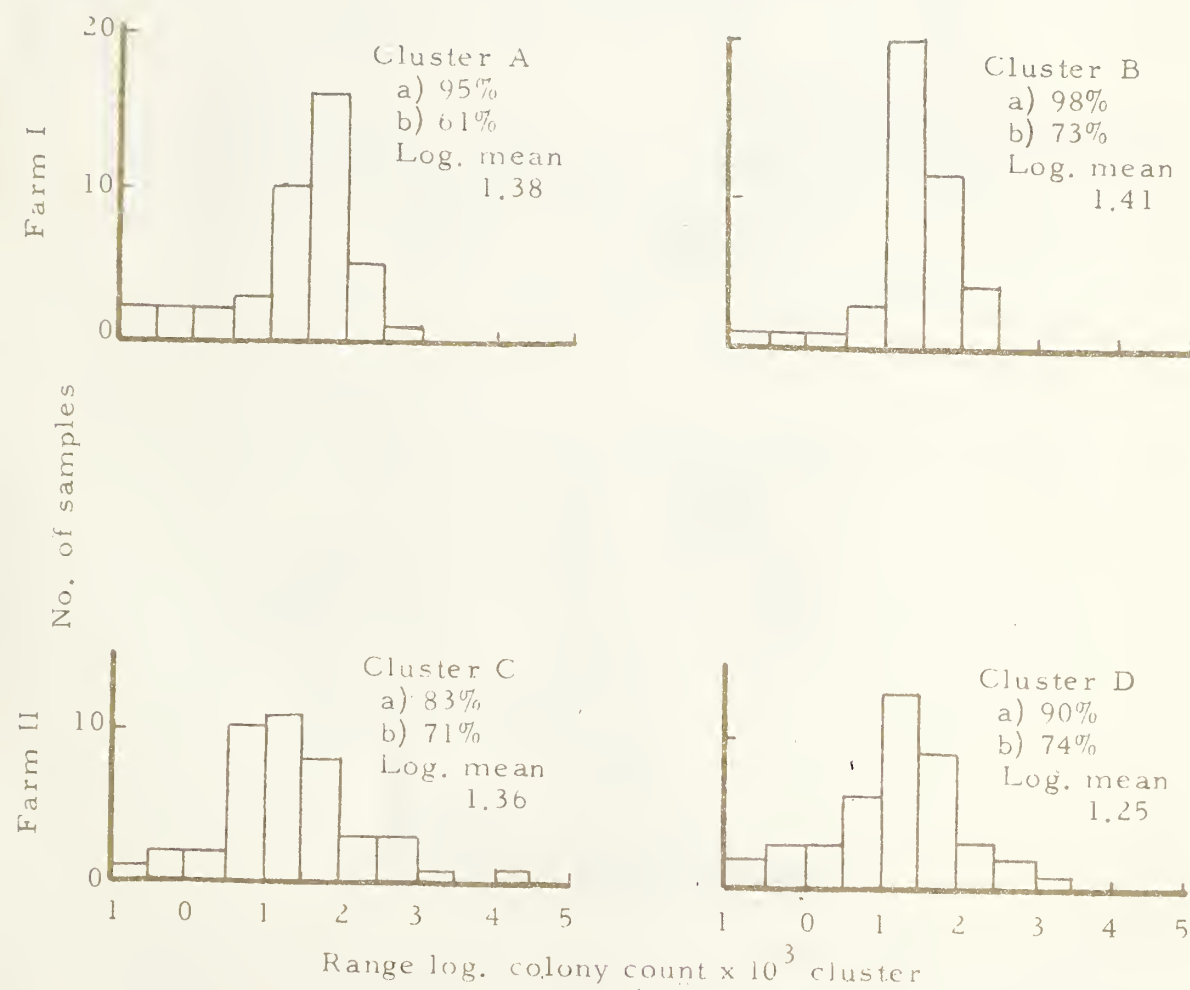


Fig. 4. Frequency distribution of colony counts of rinses of clusters from two experimental farms. a) percentage of rinses below 200,000 cluster, b) percentage of rinses below 50,000 cluster.



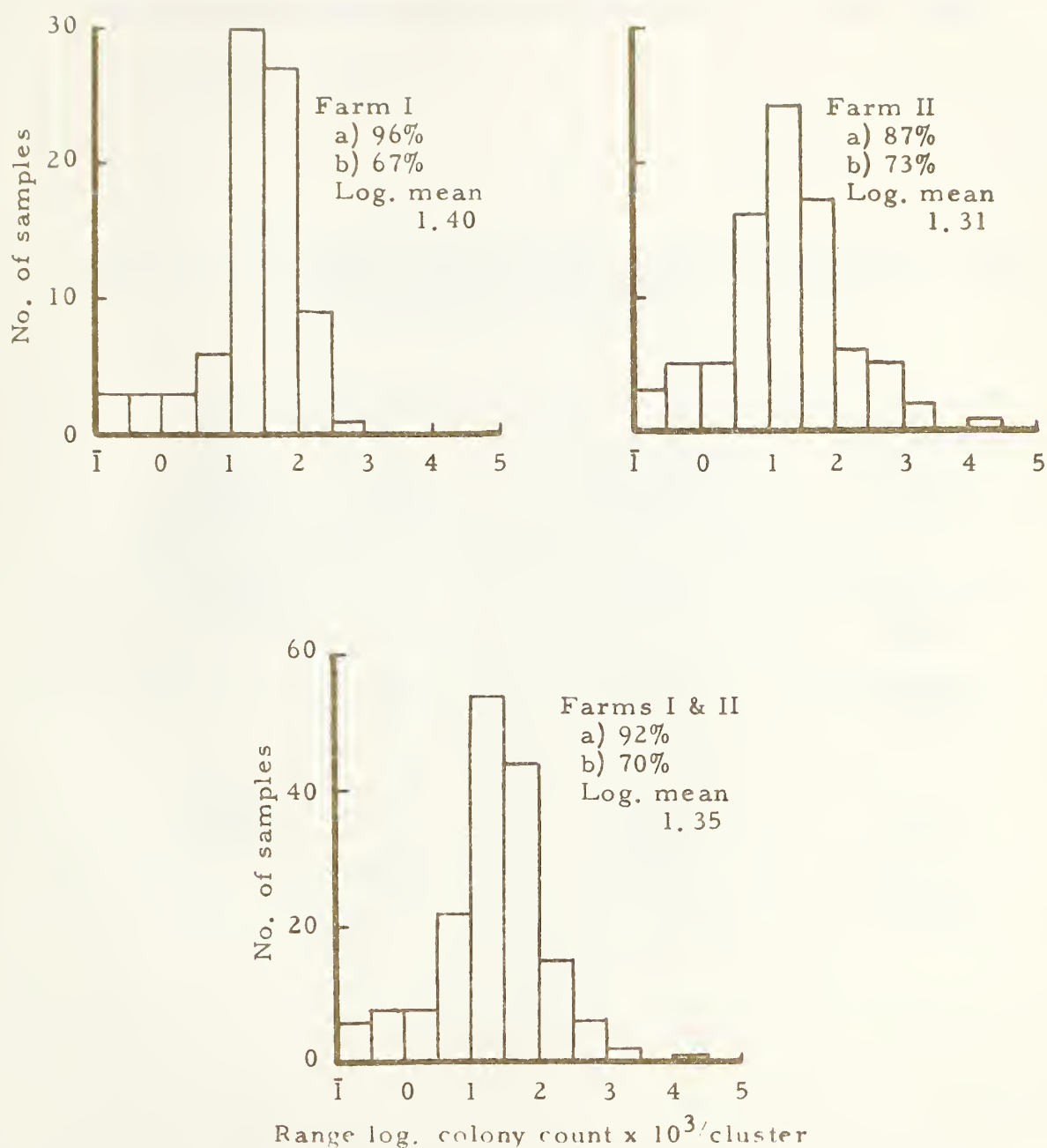


Fig. 5. Frequency distribution of colony counts of rinses of clusters for each farm and for the two farms taken together. a) percentage of rinses below 200,000/cluster, b) percentage of rinses below 50,000/cluster





Bacteriological examination of water supply. The results in Table 13 were obtained from an examination of the water supply from the two farms.

Table 13. A simple bacteriological analysis of the water supply of the two experimental farms

Date of sample	Farm	Standard Plate Count/ml	Approximate coliform content
12 Sept. 1960	I	16,000	not less than 10/ml
"	II	7,100	less than 1/ml
21 Oct. 1960	I	23,000	10/ml
"	II	3,500	less than 1/ml



## II Other investigations and results

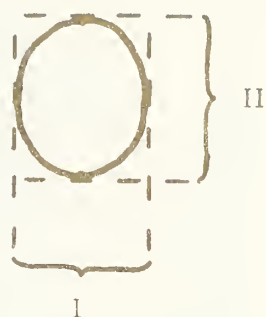
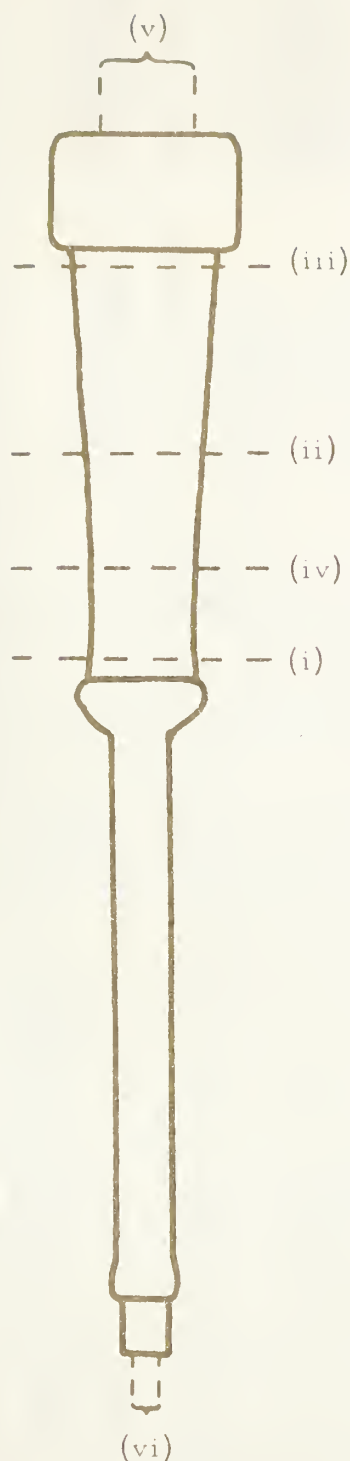
### (i) Examination of rubberware

At the commencement of the trial, all rubberware was replaced with new. Two liners were replaced on Farm I after four months of the trial owing to accidental damage or to faults in manufacture. On Farm II, liners were replaced after 10 months<sup>†</sup> use owing to the fact that they tended to slip off the clawpieces. Shortly afterward, those on Farm I were also replaced for the same reason.

Two representative liners were taken from each of the following five groups: A, new liners; B, liners from Farm I after 4 months<sup>†</sup> use with Immersion Cleaning; C, liners from Farm II after 10 months<sup>†</sup> use with Immersion Cleaning; D, liners from Farm I after 10 - 12 months<sup>†</sup> use with Immersion Cleaning, and E, liners from Farm II which had been treated by another method of cleansing and had been discarded before the trial of Immersion Cleaning was started. The liners were measured for length, width of barrel at (i) narrow end, (ii) centre, (iii) wide end, (iv) narrowest point; diameter of teat opening, and internal diameter of end which fits onto clawpiece. The positions at which the liners were measured are illustrated in Fig. 6. The appearance of the external surface was noted and then the liners were cut open lengthwise and the internal surfaces observed.

The measurements and observations on the liners are given in Table 14. Table 15 compares the degrees of distortion of the liner barrels for the different liners.





- (i) Diameter of barrel at narrow end.
- (ii) Diameter of barrel at centre.
- (iii) Diameter of barrel at wide end.
- (iv) Diameter of barrel at most flattened point.
- (v) Diameter of teat opening.
- (vi) Diameter of end which fits onto clawpiece.

- I    Narrowest dimension
  - II    Widest dimension
- } at same cross-section.

Fig. 6. Diagrams to show where the measurements of liners given in Table 14 were taken





Table 14. The effect of different treatments on the condition of rubber teat-cup liners

Liner	Length	Width of barrel at:										(vi)	Appearance of external surface	Appearance of internal surface
		(i)		(ii)		(iii)		(iv)		(v)				
		I	II	I	II	I	II	I	II					
A	27.6 27.7	2.82 2.82	2.82 2.82	2.90 2.92	2.91 2.93	3.20 3.23	3.21 3.24	2.82 2.80	2.82 2.80	2.55 2.53	0.87 0.88	Shiny and smooth "	Shiny and smooth "	
B	28.1 28.1	2.77 2.78	2.78 2.84	2.78 2.74	2.99 3.04	3.24 2.19	3.24 3.19	2.58 2.70	2.97 2.98	2.56 2.55	0.92 0.91	Fairly shiny "	Upper part of barrel fairly rough but clean and free from deposit "	
C	28.2 28.2	2.78 2.77	2.78 2.80	2.77 2.80	3.02 3.05	3.18 3.25	3.28 3.28	2.66 2.67	2.91 2.98	2.53 2.53	0.88 0.91	Shiny surface, new looking "	"	
D	28.2 28.2	2.78 2.78	2.80 2.82	2.68 2.72	3.13 3.10	3.17 3.16	3.24 3.26	2.58 2.64	3.07 3.05	2.56 2.56	0.91 0.91	" "	" "	
E	28.6 28.5	2.76 2.77	2.78 2.79	2.61 2.72	3.20 3.04	3.31 3.27	3.37 3.40	2.58 2.62	3.12 2.95	2.58 2.81	0.94 0.92	Dull surface, surface rough and flecked "	Much deposit, which is very difficult to remove	
A	New liners.										(i)	narrow end.		
B	Farm I after 4 months Immersion										(ii)	centre.		
C	Cleaning.										(iii)	wide end.		
D	Farm II after 10 months "										(iv)	most flattened point.		
E	Farm I after 10-12 months "										(v)	diameter of teat opening.		
	Farm II, liners 3-4 months old, cleansed by another chemical method.										(vi)	internal diameter of end which fits onto clawpiece.		
											I	narrowest dimension	at same cross-section.	
											II	widest dimension		
												Measurements in cm.		



Table 15. The effect of different treatments of teat-cup liners expressed as arbitrary numerical values

Treatment of liner	Differences between measurements I and II of the barrels of liners, to compare the degrees of distortion, at position:			
	(i)	(ii)	(iii)	(iv)
A	0 0	0.01 0.01	0.01 0.01	0 0
B	0.01 0.06	0.11 0.30	0 0	0.39 0.28
C	0 0.03	0.25 0.25	0.10 0.03	0.25 0.31
D	0.02 0.04	0.45 0.38	0.07 0.10	0.49 0.41
E	0.02 0.02	0.59 0.32	0.06 0.13	0.54 0.33
A	New liners.			
B	Farm I after 4 months Immersion Clnng.			
C	Farm II after 10 mos. "			
D	Farm I after 10-12 mos. "			
E	Farm II, liners 3-4 mos. old, cleansed by another chemical method.			
		(i)	narrow end.	
		(ii)	centre.	
		(iii)	wide end.	
		(iv)	most flattened point.	
		I	narrowest dimension	} at same cross-section
		II	widest dimension	

1. The first part of the document is a list of the names of the persons who have been appointed to the various positions in the organization.

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5. The fifth part of the document is a list of the names of the persons who have been appointed to the various positions in the organization.

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Plate 5 compares the lengths of liners which have received the five treatments, A, B, C, D and E. Plate 6 shows the degree of distortion of the teat openings of the five types of liner.





Plate 5. The effect of different treatments on the length of rubber teat-cup liners.

A, New liners; B, Liners from Farm I after 4 months' Immersion Cleaning; C, Liners from Farm II after 10 months' Immersion Cleaning; D, Liners from Farm I after 10-12 months' Immersion Cleaning; E, Liners from Farm II cleansed by another chemical method, 3-4 months' old.





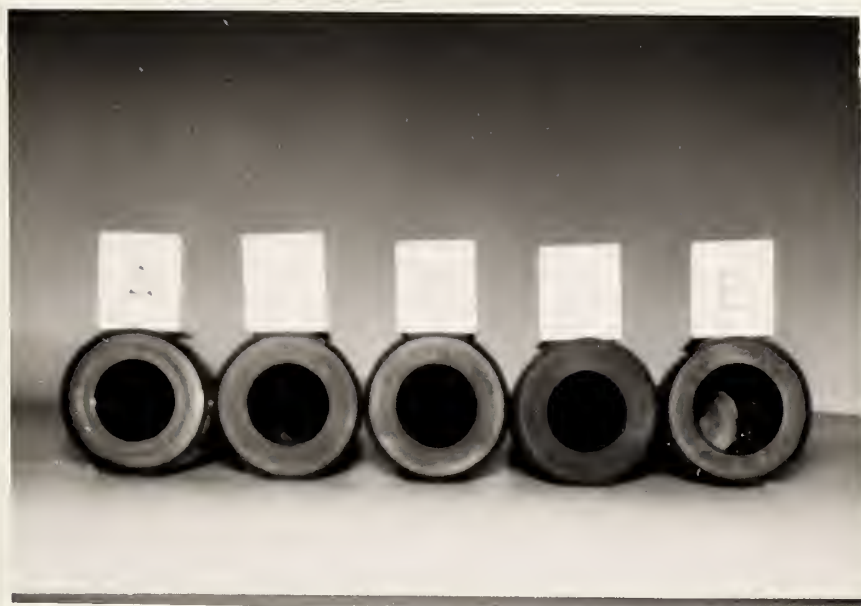


Plate 6. The effect of different treatments on the diameter of the teat openings of rubber teat-cup liners.

A, New liners; B, Liners from Farm I after 4 months<sup>†</sup> Immersion Cleaning; C, Liners from Farm II after 10 months<sup>†</sup> Immersion Cleaning; D, Liners from Farm I after 10 - 12 months<sup>†</sup> Immersion Cleaning; E, Liners from Farm II cleansed by another chemical method, 3 - 4 months<sup>†</sup> old.



(ii) Retaining tension on liners during immersion

It was decided to see whether releasing the tension on the liners before immersion in the solution was necessary. For a short while the liners were allowed to remain in the position in which they are used for milking, i.e. the liners were not freed from the ends of the shells and the tension was allowed to remain on the liners. Two of the short air tubes were allowed to remain on the clawpieces while the other two remaining on the shells were cross-connected to the shells from which the air tubes had been removed, thus making as water-tight as possible the cavity between the liners and the shells.

After a very short period of use in the above manner, it was found that the solution had leaked into the cavity between the liners and the shells and was being sucked up the long air tube to the pulsator and was interfering with the proper functioning of the pulsator and so this practice was discontinued.

(iii) Titration of caustic soda solution at the end of the months' use

Occasionally samples of the caustic soda solution were taken for titration. These were titrated against 0.50N HCl (which is equivalent to a 2% solution of sodium hydroxide) using bromthymol blue as indicator.

It was found that the solution at the end of 4 or 5 weeks' use was approximately 2%. The five samples taken ranged from 1.8% to 2.24% sodium hydroxide, with a mean of 2.0%.



(iv) Titration of water hardness

When the trial was commenced, the immersion solution was made using 6 ozs EDTA/12 gal of solution. This was the amount recommended for very hard water by Thiel et al. (1956) and this amount was used to ensure that no trouble was encountered with build-up of deposits. However, samples of the water from the two farms were analyzed and the hardness of both was found to be very low (Farm I, 20 p/m; Farm II, 10 p/m). In addition to this analysis which was carried out by the Provincial Analyst, samples of the water were titrated directly using EDTA, and it was calculated that both samples of water would require less than 0.2 ozs EDTA/12 gal of solution to prevent deposits from the water alone. It was decided that 2 ozs EDTA/12 gal of solution would be sufficient to take care of the minerals added by milk residues left on the equipment, and so after five months of the trial, only 2 ozs EDTA was used instead of 6 ozs. Since there was never any appreciable visible build-up of deposit on the equipment, it was concluded that this amount of EDTA was sufficient.

(v) Temperature of the milk-house and immersion solution

During the last 8 months of the trial, a maximum-minimum thermometer, located in the milk-house near the immersion solution, was set the evening before the milk samples were collected. The maximum and minimum temperatures were read when the samples were collected and also the temperature of the immersion solution taken. The results are given in Table 16.

The first part of the report, which is the most important, is the one that deals with the results of the experiments. It is in this part that the author shows that the results of the experiments are in good agreement with the theoretical predictions. This is a very important result, as it shows that the theory is correct. The second part of the report is the one that deals with the discussion of the results. It is in this part that the author discusses the implications of the results and the limitations of the experiments. The third part of the report is the one that deals with the conclusions. It is in this part that the author summarizes the main findings of the report and gives some suggestions for further work.

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Table 16. Maximum and minimum temperatures of the milk-house and temperature of the immersion solution ( $^{\circ}\text{F}$ )

Date	Farm I		Farm II	
	Temperature			
	range of milk-house	of solution	range of milk-house	of solution
1960				
7 Nov.	36 - 54	-	40 - 73	46
12 Dec.	-	-	47 - 79	52
1961				
10 Jan.	50 - 63	45 ?	42 - 82	55
6 Feb.	53 - 63	53	-	53
6 Mar.	32 - 44	32	43 - 80	43
12 Apr.	49 - 52	49	39 - 58	52
8 May	32 - 52	44	50 - 88	52
Mean		45		50



(vi) Rinsability trials

The farmers found that the rubber parts and particularly the short air tubes tended to slip off the clawpieces during milking, especially when the immersion solution was newly made (i.e. at the beginning of the month's use).

It was thought that the addition of a chemical substance to the chlorinated rinse used before milking might help to more thoroughly remove the residual caustic soda on the rubber surface and thus prevent slipping. Caustic soda solutions are very slippery and are difficult to rinse off. And so the following screening tests for chemicals with good rinsing properties were conducted.

a) Rinsability trials with distilled water for different periods of time.  
Square inch samples of rubber were cut from the barrels of new (N) liners and liners used with Immersion Cleaning for 10 months (U). Two samples of new rubber were attached to each of 6 pieces of stainless steel wire and likewise 2 samples of used rubber were attached to 6 different pieces of wire. These were labelled: 1N, 2N, . . . . 6N, and 1U, 2U, . . . . 6U respectively.

Samples 1 to 5 were immersed in a 3% solution of caustic soda for 24 hr and samples 6 were immersed in distilled water for 24 hr. Each wire was then removed individually from the solution and the rubber samples allowed to drain for 10 sec, the samples then being touched on the rim of the beaker to remove any adhering drops of solution. The rubber samples were then dipped into an individual beaker



containing 200 ml of distilled water and plunged 5 times. After being allowed to remain in the distilled water for a given period of time, the samples were again plunged 5 times, removed from the water, allowed to drain for 10 sec and touched on the rim of the beaker to remove any adhering drops. Then one of the two samples of rubber was removed from the wire and placed in 20 ml of distilled water. The other sample of rubber was felt to see how slippery it was. The 20 ml of distilled water containing the rubber sample was then titrated with 0.001 N HCl using bromthymol blue indicator.

The treatments were:

- 1 Transferred directly from the caustic soda solution to 20 ml distilled water, i.e. not rinsed.
- 2 Rinsed in 200 ml distilled water for 20 sec.
- 3 Rinsed in 200 ml distilled water for 1 min.
- 4 Rinsed in 200 ml distilled water for 2 min.
- 5 Rinsed in 200 ml distilled water for 5 min.
- 6 Control. Samples kept in distilled water instead of caustic soda solution but rinsed and titrated as other samples.

The results are given Table 17. It can be seen that caustic soda is rinsed off the surface of new rubber very easily and quickly with distilled water. With used rubber, alkalinity remained on the rubber after rinsing, but this was slowly removed by soaking in water. The surface of the used rubber was very slippery and soapy and on removal from the caustic soda solution was covered with a layer of soap. Even after neutralization, some of the soap remained in the rubber and, if the neutralized solutions were allowed to stand for a while, they gradually



Table 17. The effect of the time of rinsing in distilled water on the amount of alkalinity remaining on rubber which has been soaked in 3% caustic soda solution for 24 hr

Rinsing time (min)	New rubber		Used rubber	
	ml 0.001 N HCl to neut- ralize alkal- inity on 1 sq.in. rubber	Slipperi- ness	ml 0.001 N HCl to neut- ralize alkal- inity on 1 sq.in. rubber	Slipperi- ness
0	31	slippery	78	layer of white soapy material on both sides of sample
0.33	0	slightly slippery	7	soapy
1.0	0	"	7	"
2.0	0	not <sup>11</sup> slippery	4	slightly soapy
5.0	0	"	3.2	"
Control	0	"	0.2	not soapy





turned alkaline again, indicating that alkalinity was slowly being released from the rubber.

b) Rinsability trials with different compounds for 1 min. Different rubber samples were used. The same technique as used before was followed, except that the rinsing time was fixed at 1 min and solutions of the following compounds were used:

- 1 Chlorinated trisodium phosphate, 0.64 g/200 ml (= approximately 60 p/m available chlorine).
- 2 Sodium hexametaphosphate, 0.25% plus Chloramine T at approximately 60 p/m available chlorine.
- 3 Sodium metasilicate, 0.25% plus Chloramine T.
- 4 'Pennsan' (an acid sanitizing agent), 2.47 ml/200 ml (approximately 200 p/m).
- 5 Tetrasodium pyrophosphate, 0.25% plus Chloramine T.
- 6 Distilled water.

Chloramine T, at approximately 60 p/m, was added to solutions not having an appreciable disinfectant quality at the concentrations used.

The results in Table 18 were obtained. Again only the used rubber retained any alkalinity. Some of the compounds used for rinsing are themselves alkaline, and carry over of this solution would contribute a little to the residual alkalinity detected.

There appears to be little difference between the different compounds tested. In this limited test, tetrasodium pyrophosphate proved slightly superior to the other compounds. This work is being continued.



Table 18. The effect of rinsing in solutions of different compounds for 1 min on the amount of alkalinity remaining on rubber which has been soaked in 3% caustic soda solution for 24 hr

Solution	New rubber		Used rubber	
	ml 0.001 N HCl to neut- ralize alkal- inity on 1 sq.in. rubber	Slipper- iness	ml 0.001 N HCl to neut- ralize alkal- inity on 1 sq.in. rubber	Slipper- iness
1	0.15	not slippery	19.4	soapy
2	0	" "	17.5	"
3	0.45	" "	21.0	"
4	0	" "	18.0	"
5	0.20	" "	16.5	"
6	0	" "	24.0	"

- 1 Chlorinated trisodium phosphate, 0.64 g/200 ml.
- 2 Sodium hexametaphosphate, 0.25% plus Chloramine T.
- 3 Sodium metasilicate, 0.25% plus Chloramine T.
- 4 'Pennsan', approximately 200 p/m.
- 5 Tetrasodium pyrophosphate, 0.25% plus Chloramine T.
- 6 Distilled water.



B. TRIALS WITH SUSPENDED BUCKET MILKING MACHINES  
(WITH TEAT-CUP INFLATIONS ATTACHED DIRECTLY TO  
THE BUCKET LID.) UNIVERSITY FARM.

The use of this type of milking machine required another design of immersion basket. With this type of machine the four teat-cups are attached by the ends of the inflations directly to the lid which fits onto the bucket, i.e. there is no teat-cup cluster as there is with the type of machine in which there is a long milk tube. The main piece of equipment that requires to be treated by Immersion Cleaning is the lid with the four teat-cups attached.

An immersion basket was designed so that the teat-cup lid units could be hung vertically to prevent air-locks when the equipment was lowered into the solution. The design is simple and details of the basket can be seen in Fig. 7.

Four milking units were used, 2 for the Jersey herd and 2 for the Holstein herd, so the basket was designed to accept the four teat-cup lid units. Since no long milk tubes are used with this type of machine, the spiral rack to accept these in baskets for the other type of unit was not necessary.





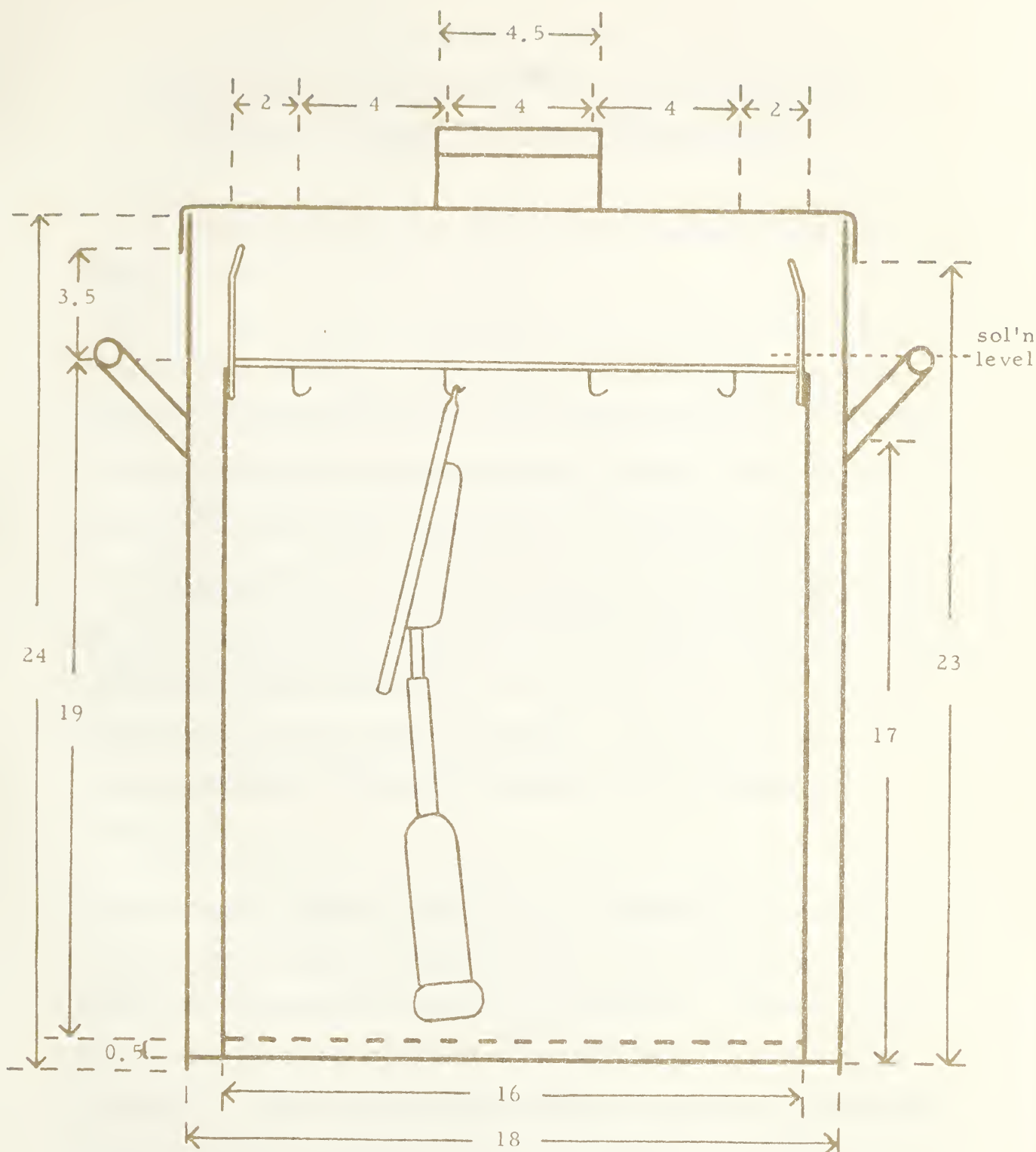


Fig. 7. Diagram of the immersion basket in the bin to take units of the suspended bucket type milking machines (Measurements in inches).



## Suggested daily routine for use of the method of Immersion

### Cleaning with suspended bucket milking machines

The instructions given to the herd-man at University Farm were as follow:

"After milking. Remove the pulsators from the lids but leave the short air tubes attached to the shells. Brush off any dirt on the outside of the equipment with a wet brush. Rinse clusters and lids with water (or hypochlorite solution used before milking and left in the wash-up trough) to remove any milk residues and remaining dirt.

Extend liners past the ends of the shells. Remove gaskets and any loose parts and place in the bottom of the immersion basket. Hook lids with teat-cups attached onto bar across the immersion basket so that the teat-cups hang freely downwards. Lower the basket into the solution in the bin. Replace lid and leave until just before next milking time.

Before milking. Make up a few (5) gal of hypochlorite solution (50 p/m) in cold or warm water in wash-up trough. Lift the basket out of the solution and let equipment drain for a few seconds. Place the basket on the floor and, if desired, hose down the outside of the equipment with water. Lift the equipment into the prepared hypochlorite solution. Rinse thoroughly. Pull liners back onto the shells and assemble ready for milking. Leave the solution in the wash-up trough for rinsing after milking."



The solution was changed monthly and the quantity of solution necessary to cover the equipment was 16 gal. This required approximately 4-1/2 lbs of commercial caustic soda to give approximately 3%, and 4 ozs EDTA was used. The equipment was taken apart and brushed once a month and the metal parts acid-treated also once a month.

As in the other trial, the equipment which was not treated by Immersion Cleaning was washed in detergent and sanitized chemically

Sampling was commenced in February 1960. At this time the equipment was dismantled daily and brushed in detergent solution and sanitized chemically. The liners were stored between milkings in a crock containing 0.5% caustic soda solution. Immersion Cleaning was commenced in September 1960.

#### Changes found necessary in the recommended technique

With this type of machine it was found that during milking, dirt became lodged and dried on the lids under the metal protrusions on which the liners fit, owing to the cows kicking the machines while being milked. This was difficult to remove with a wet brush and so, after milking, the operators soaked the whole assembly in the wash trough for 30 min to loosen the dirt. This was then removed by brushing and rinsing before the units were immersed in the caustic soda solution.

The operators found that it was difficult to remove moisture from the short air tubes (i. e. the air tubes from the shells directly to the





pulsators). This moisture was sucked into the pulsators during milking and interfered with their operation. With this type of machine the pulsator is connected directly to the short air tubes attached to the shells. And so the operators suggested not protruding the liners past the ends of the shells when the equipment was immersed, and in order to make the cavity between the liners and the shells watertight, they suggested removing two of the short air tubes along with the pulsator, the other two being left on the shells and cross-connected to the shells from which the tubes had been removed. Thus the space between the liners and shells would be completely closed off. This was tried and, before milking, the liners were protruded to check whether any liquid had seeped into this cavity. It was found that no moisture was present and so this practice was continued. In addition to being satisfactory, it saved time in the operation by elimination of protruding the liners.

The design of the teat-cup shell with this type of machine is such that the liner is caused to collapse in one plane only throughout milking. This is achieved by two ridges running lengthwise along the shell. Normally where the equipment is taken apart daily there is a great chance that a liner will not be put back into a shell in the same position. However, where the equipment is not dismantled daily, it was thought that the liners would be weakened and distorted by collapsing always in one way. And so each liner was rotated approximately a quarter of a turn each day to attempt to prevent this.

The teats of the first six cows became sore and the operator





maintained that this was caused by residual caustic soda on the liners. And so, after removal from the caustic soda solution, the equipment was soaked for 30 min in the calcium hypochlorite rinse.

In order to determine the amount of caustic soda left on the liners, a cluster was rinsed with 200 ml of distilled water after soaking for 30 min in the hypochlorite solution. The rinse was titrated with N/50 sulphuric acid using methyl orange as indicator and it was found that 25 ml of the rinse was equivalent to 0.2 ml of the acid. From this it was found that the total amount of alkalinity (calculated as sodium hydroxide) removed from the cluster was 0.00128 g.

It was concluded that if residual caustic soda did cause sore teats, soaking for 30 min left a negligible amount on the equipment which probably would not cause sore teats.



## I Bacteriological Methods

### Sampling.

Weekly samples of milk were taken from one of the buckets and from the bulk tank. The university herd was composed of a Jersey and a Holstein herd, two milking units being used for each herd. As one of the milk samples was taken directly from the bucket, this meant that the milk of only one cow was sampled. In order not to sample milk from the same cow continually, the following routine was adopted. One week the milk from the first Holstein to be milked with the first machine was sampled. The next week the first Holstein to be milked with the second machine applied was sampled. The third week the first Jersey to be milked was sampled and the fourth week the second Jersey to be milked, thus constituting a four-week cycle. The samples were always taken on a day when the bulk tank was empty and a sample of the bulked milk was taken as soon as there was sufficient milk in the tank for a sample to be taken.

On the same occasion that the milk samples were taken, one of clusters was rinsed. For two consecutive weeks, Holstein clusters were rinsed and the following two weeks, Jersey clusters. Rinses were taken by detaching each liner separately from the clawpiece attachment on the lid and pinching the end of the liner. The neck of the milk bottle containing the 500 ml of sterile buffered rinse was fitted into the end of the liner and the bottle and liner inverted so that the teat-cup



became filled with rinse. This was allowed to drain back into the rinse bottle and the process repeated. Each liner was rinsed twice with the rinse solution.

#### Laboratory examination of samples.

A Standard Plate Count, Methylene Blue Test and a simple Coliform Test were applied to the milk samples, and a Standard Plate Count only to the rinses.

### Results

#### Milk samples.

The results of analysis of the milk samples were extremely good, the geometric mean for the plate count on bucket samples being 520 colonies/ml, and that for the plate count on bulk tank samples being 1,700/ml, before Immersion Cleaning was commenced. When Immersion Cleaning was used, the results were also very good, the geometric mean for the plate count on bucket samples being 970/ml and that for bulk tank samples being 760/ml. All samples had a methylene blue reduction time of more than 7 hr and in the majority of cases, coliform organisms were absent in 1 ml of the milk.

#### Cluster rinses.

The rinses of clusters were also satisfactory in most cases. The geometric mean of rinses before Immersion Cleaning was used was 13,500/cluster (for 29 samples) and when Immersion Cleaning was used it was 17,300/cluster (for 12 samples).





## DISCUSSION OF IMMERSION CLEANING RESULTS

On Farms I and II the geometric means for all milk samples were below 37,000/ml. The means for Farm II, where the samples were refrigerated, were generally lower than those of Farm I where the samples were not refrigerated. Even though they were not refrigerated, the samples from Farm I were generally satisfactory, the geometric mean for all samples from this farm being 29,000/ml. The mean for the PM sample from Farm I was the highest. This was the sample receiving the most adverse treatment, being a sample of evening milk which had been allowed to remain at the temperature of the milk-house until it was collected approximately 20 hr afterwards. Even so, the mean for this sample was 37,000/ml and this is well below the commonly accepted standard for Grade A raw milk to be pasteurized of 200,000/ml. The distribution of colony counts of milk samples can be seen from Fig. 2 and Fig. 3. The percentage of samples having a colony count of less than 200,000/ml is high and the samples having counts of greater than this value are mostly samples which were kept at the temperature of the milk-house before collection during hot weather.

Similarly, the majority of the methylene blue reduction times for the samples were satisfactory.

As can be seen from Table 7, samples of milk taken from the bulk tank showed generally higher coliform contents than the other



milk samples. This suggests possible contamination with particularly this type of organism from the strainer or bulk tank, or during the milking procedure, since the morning and evening samples were taken always from the first bucket or pail of milk. Colony counts are also higher for bulk tank samples than for the other samples, with the exception of the PM sample from Farm I.

The arbitrary standard of a colony count of 50,000/sq ft has been suggested by Clegg & Hoy (1957) and Clegg et al. (1959) for milking equipment in a satisfactory bacteriological condition. Even this degree of contamination would increase the colony count/ml of milk very little and counts occasionally higher than this could be tolerated. The milk-contact surface area of a cluster assembly is taken as being approximately 1 sq ft.

The geometric mean for all rinses was 22,500/cluster, which is satisfactory. The means for Farm I were slightly higher than those for Farm II as can be seen from Table 11, but these were still satisfactory. The distribution of the rinse counts are shown in Figs. 4 and 5. Taking all the rinses together, 70% were 50,000/cluster or less and 92% were 200,000/cluster or less.

Milk samples from University Farm were exceptionally good, both with Immersion Cleaning and with the method used before Immersion Cleaning was used, as were the cluster rinses. Of the 143 milk samples tested, only 5 had a colony count of greater than 10,000/ml which is excellent. Of these 5 occasions, 1 was when Immersion Cleaning was being used.



Tables 14 and 15 show that while all of the used liners became somewhat mis-shapen, those being treated by Immersion Cleaning were considerably less affected than those treated by daily brushing in detergent and wet storage. Liners E (not treated by Immersion Cleaning) were only 3 - 4 months old when they were discarded, whereas liners C and D were used for 10 or more months with Immersion Cleaning. The appearance of the inside surface of the liners showed a remarkable difference between the two treatments. While there was a slight roughness of the rubber inside the liners treated with Immersion Cleaning, there was no build-up of deposit as there was with liners E. The slight roughness may be due to removal by caustic soda of the fat which is able to penetrate the rubber during milking. There was no complaint from the operators about this slight roughness and it caused no ill effects on the cows, except possibly in the case of the University Farm. The herd-man at the University Farm maintained that since Immersion Cleaning was used, the teats of some of the cows became sore. He blamed carry over of caustic soda as the cause of this and possibly the roughness of the liners in conjunction. The liners used with this type of machine were of a different type from those used with the type of milking machines on Farms I and II. The liners at the University Farm did become rough after a short period of use. It is very doubtful whether the roughness of the surface would compare to the roughness of a calf's tongue, and this roughness alone most probably would have no ill effects on the teats of healthy cows.

With the suspended bucket milking machines, where the pulsator





is very near to the teat-cups, it is more important to ensure that no moisture is present between the liners and the teat-cup shells, otherwise this is soon sucked the very short distance to the pulsator and interferes with the pulsator's proper functioning. Leaving the liners under tension and cross-connecting 2 of the short air tubes to the shells from which the tubes have been removed provides a sufficiently water-tight seal to prevent solution seeping into the cavity between liner and shell during immersion. With bucket milking machines with long milk tubes, the presence of a small amount of moisture in the air tubes is less important since this has to travel a considerable distance to the pulsator. It has been found satisfactory to release the tension on the liners during immersion, for with this type of machine the seal between liner and shell is not sufficiently water-tight to prevent seepage of solution into the cavity between liner and shell. Before assembly of the machines for milking, any liquid retained by the deep skirt of this type of liner is tipped out to prevent its being sucked up to the pulsators.





## CONCLUSIONS

That the method of Immersion Cleaning, adapted for use with bucket-type milking machines, produces satisfactory results is evident from the trials on the 3 farms in Canada.

The farmers agreed that this method saves considerable time and labour and is generally more economical than daily dismantling of the milking machines, brushing in detergent solution and sanitizing, even though it is necessary to treat parts of equipment not able to be cleansed by Immersion Cleaning, separately. The life of the rubberware is considerably lengthened.

On the farms using Immersion Cleaning in these trials, the milk-houses where the Immersion Cleaning equipment was kept were never very cold. During the first winter of trial A, both of the milk-houses were heated, and during the second winter, the milk-house on Farm II was heated by a heater whereas that on Farm I was warmed by leaving the door between the milk-house and the milking barn open continuously. Unfortunately, temperatures of the milk-houses and immersion solutions were not taken from the commencement of the trial. However, temperatures taken during the second winter revealed that the temperature of the immersion solution was approximately 45°F on Farm I and approximately 50°F on Farm II.

Since the efficiency of a disinfectant is dependent upon temperature,



it was thought that a 3% solution of caustic soda might not produce satisfactory bacteriological results if the temperature of the solution was very low (i.e. around freezing point) which is likely to occur in milk-houses on some Canadian farms not using an efficient heating system during very cold weather. In order to determine the bactericidal efficiency of different concentrations of caustic soda at different temperatures, disinfection studies, reported in part II of this work, were undertaken.



## PART II

### THE DISINFECTANT PROPERTIES OF SODIUM HYDROXIDE SOLUTIONS

#### INTRODUCTION

#### METHODS OF ASSESSING THE BACTERICIDAL EFFICIENCY OF A DISINFECTANT.

There are two main types of method used for estimating the efficiency of a disinfectant.

1. Estimation of the total extinction time. This method tests only for the presence or absence of viable organisms in a culture which has been subjected to a disinfectant action, i.e. the time required to kill the whole population of organisms is estimated.
2. Determination of the count of surviving organisms throughout the course of the disinfection. With this type of method, the number of surviving organisms can be plotted against time and the shape of the time-survivor curve is able to furnish valuable information on the disinfection process.

In addition to these two main methods, other methods of estimating the efficiency of antibacterial substances are used for special purposes, but they were not considered suitable for the present studies.





## COMPARISON OF THE TWO MAIN METHODS FOR ESTIMATING THE EFFICIENCY OF A DISINFECTANT.

The resistances of individual cells in a culture vary greatly. Withell (1942) pointed out that if the individual resistances to disinfection of cells in a population were plotted against time in the form of frequency histograms, it was found that a normal distribution was not obtained. However, if the resistances were plotted against the logarithm of time, frequency histograms were obtained which were near to normal distributions. He suggested then that the resistances of cells in a population are distributed logarithmically.

There is thus a small proportion of cells which possess very much greater resistances than the rest of the population. With methods involving the detection of total extinction time, it is the survival of these highly resistant cells that is estimated, and this time may be considerably greater than the time taken to destroy the majority of the population. With methods in which time-survivor curves are obtained, it is possible to detect the time taken to kill a certain proportion of the population, say 99%, 99.9%, etc. and this time may give more useful information than the time required to kill the few highly resistant organisms.

Cook & Wills (1954) maintained that coefficients of disinfection based on end-point methods are of comparable reliability with those based on counts of surviving organisms. But for some purposes, as



for the present, more information is furnished by methods producing time-survivor curves, than methods involving the detection of total extinction times, since in dairy practice, disinfection processes are rarely 100% effective and methods not producing complete sterility can be tolerated. And so it is more useful to know the conditions necessary to produce say 90%, 99% or 99.99999% destruction.

Towards the end of the disinfection period, when very few of the organisms remain, it is a matter of chance whether a viable organism is picked out of the disinfection medium. This may give rise to uncertain end-points with methods concerned with total extinction time, especially if the rate of disinfection is slow, i.e. the time when few organisms are surviving is long.

Many workers have obtained time-survivor curves for various disinfectants under different conditions. Withell (1942) gave an extensive list of the types of curves and conditions of experiments of many previous workers. The types of curve most frequently obtained when the logarithms of the number of surviving organisms were plotted against time were curves generally (i) exponential, (ii) sigmoid, and (iii) concave. Occasionally convex curves were reported but these most probably were the first part of a sigmoid curve.

Withell (1942) pointed out that the type of curve obtained when the logarithm of the percentage of organisms surviving is plotted against time depended partly upon the standard deviation of the resistances of the cells in a population. If the standard deviation is small,



most of the organisms will have resistances close to the mean resistance, i.e. there will be relatively few individual cells with low and relatively few with high resistances. In this case, when the logarithm of the percentage of cells surviving is plotted against time, there is likely to be a lag followed by an exponential curve and finally a slight tailing off. If the rate of disinfection is rapid, the lag period may take place so rapidly that it is undetected and only an exponential curve or an exponential curve which tails off may be apparent. If the standard deviation of resistances is large, i.e. there is a spread of resistances, there being many individual cells with low and many with high resistances, the resultant time-log. survivor curve will be sigmoid.

However, besides the spread of resistances of individual cells, many other factors influence the results obtained in a disinfection study.

It has been reported by many workers that the medium in which the organisms are grown affects the general resistance of the population (e.g. Charney et al., 1951; Curran, 1952; Schmidt, 1955; Amaha & Ordal, 1957).

Bacteria surviving a disinfection treatment are more exacting in their nutritive requirements and special enrichment media are necessary for their accurate enumeration (e.g. Schmidt, 1955; Franklin & Clegg, 1956; Williams et al., 1957a, b; Jacobs & Harris, 1960; Amaha & Ordal, 1961). The enrichment medium necessary to recover damaged cells may differ with the species of organism,





the nature of the lethal agent and the extent of the disinfectant action (Schmidt, 1955). This may be due to the presence of toxic substances present in usual media which are removed or destroyed by the inclusion of certain other substances (Jacobs & Harris, 1960). Special media are necessary to detect the survival of spores particularly after a disinfection treatment. This is partly due to the fact that not all viable spores germinate on normal media. It is possible that the presence of certain substances in the medium inhibit germination. Enrichment media used for the detection of spores surviving a disinfection treatment then may have a two-fold purpose, (1) to stimulate germination, and (2) to provide substances necessary for the survival (or revival) of the damaged cells.

The experimental technique itself is, of course, important in obtaining satisfactory results. Even small, somewhat less obvious factors may influence the accuracy of the results. Wills (1937) pointed out the following important factors contributing to error in the estimation of the number of organisms surviving a disinfection treatment:

1. The length of time the treated suspension is allowed to stand at room temperature before dilution.
2. The length of time between diluting and plating, and
3. The length of time between plating and incubating at the desired temperature, i.e., the length of time the plates are left at room temperature.

However, in disinfection studies using time-survivor curve





methods, the main concern is with drastic (logarithmic) changes in the number of organisms surviving. Slight inaccuracies in the exact arithmetic number of organisms estimated can be tolerated.

In studies on disinfection of spores, it is not practical to use a method of enumerating the number of surviving organisms in a certain number of drops from a calibrated pipette, as has been used by Wills (1937), Cook & Yousef (1953), Berry & Bean (1954), Cook & Wills (1954), Davis & Bell (1959) and many other workers who used vegetative organisms. With methods of this type it is necessary to use pipettes delivering drops of equal volume and these pipettes must be specially calibrated. Where vegetative organisms are being used it is possible to sterilize the calibrated pipettes, or at least the parts coming into contact with the bacterial suspension, simply by immersion in boiling water for a short period of time. Thus, only a few pipettes need to be used and need to be calibrated and these can be continually re-used after the residual vegetative organisms from a previous inoculation have been destroyed. With spores, however, the organisms remaining on a pipette cannot so easily be removed, and a much more severe heat treatment is required to sterilize the pipettes. Thus a very large number of pipettes would have to be used and calibrated and for this reason it is not practical to use this method with disinfection studies on spores, even though this method is a very accurate one.



## PLAN OF EXPERIMENT.

The concentration of sodium hydroxide used in Immersion Cleaning is 3%. At the end of the month's use the concentration is approximately 2%. And so in the disinfection studies it was decided to investigate the properties of these two concentrations and also concentrations either side of this range, and so four concentrations were chosen, viz: 1.5%, 2%, 3% and 5%. The temperature at which the immersion solution is used is likely to vary considerably from around freezing point to 70°F, i.e. the temperature of the solution is dependent upon the atmospheric temperature of the milk-house. The temperatures chosen for investigation were 32° (or near to this), 40°, 50°, 60° and 70°F.

It was decided to use bacterial spores as these are most resistant to the disinfection process. Also, the slower rate of disinfection with bacterial spores would make disinfection studies easier to carry out. For the preliminary trials, spores of Bacillus subtilis N.I.R.D. 736 were used and later, the more resistant spores of B. subtilis SM 761 (as used in the studies by Franklin & Clegg, 1956).

A method based on that of Williams et al. (1957a, b) was used, and this method is similar in principles to the methods used by many workers.



## EXPERIMENTAL METHODS AND RESULTS.

### Preliminary experiments

#### Preparation of spore suspension.

Production of spores. Charney et al. (1951) found that manganese was an essential element for sporulation in the genus Bacillus and that this element was sufficiently deficient in usual media for a stimulation in spore production to be observed if further traces of the element were added. Media containing manganese to stimulate spore production have been used by many workers including Williams et al. (1957a, b) and Halvorson (1957).

Approximately 100 ml of the following medium was added to each of 15 1-litre Erlenmeyer flasks stoppered with cotton wool, sterilized and allowed to solidify.

Bacto-tryptone	3 g
Bacto-peptone	6 g
Yeast Extract	3 g
Beef Extract	1.5 g
Agar	25 g
Solution containing	
0.001% Mn as MnSO <sub>4</sub>	1 ml
Distilled water to 1 litre	

pH approx. 7

This medium is similar to that used by Williams et al. (1957a) except that Bacto-peptone was used instead of Oxoid-peptone, Yeast Extract instead of Yeastrel and Beef Extract instead of Lab-Lemco.







It was found unnecessary to adjust the pH as it was very near to pH 7.

Each flask was inoculated with 1 ml of a 24-hr broth culture of B. subtilis N.I.R.D. 736 and incubated at 37°C for 7-1/2 days.

Harvesting the spores. The growth was washed off the agar surface with 5 ml of sterile distilled water, the surface being scraped with a sterile bent glass rod to remove the growth. The suspension was tipped into a sterile 16 oz screw-capped bottle containing glass beads. A further 5 ml of sterile distilled water (or more if necessary) was added to the flask to remove as much as possible of the growth.

When the growth from the 15 flasks had been collected, the suspension was shaken for 2-1/2 hr to break up clumps. On microscopic examination it was found that some clumps were still present and so shaking was continued for a further hour at a faster rate. On examination this time the suspension was found to be free from clumps. The suspension was then heated at 80°C for 20 min to destroy vegetative organisms.

Washing the spores. The suspension, relatively free from clumps was centrifuged at 1,500 revolutions/min (with a diameter of 18.5 inches from the bottom of one tube to the bottom of the opposite tube) for 1 min to throw down debris and remaining clumps. The supernatant was then decanted from the sediment. This was divided amongst 6 higher speed centrifuge tubes and centrifuged at 3,000 revolutions/min (with a diameter of 16 inches) for 40 min. The



supernatant was examined for spores and none were found so it was presumed that the majority of the spores had been thrown down. The supernatant was decanted and discarded. A small amount of sterile distilled water was added to each tube and the spores resuspended by stirring and shaking mechanically. More distilled water was added so that the centrifuge tubes were between a half and two-thirds full, and the contents mixed. They were then centrifuged at 3,000 revolutions/min for 30 min. It was found that there were still a few spores present in suspension but these were discarded with the supernatant.

The washing, centrifuging process was repeated 5 times altogether, centrifuging at 3,000 revolutions/min for 40 - 45 min each time except the second.

The washed spore suspension was adjusted to 100 ml with sterile distilled water and kept in the refrigerator in a stoppered bottle.

#### Selection of medium for enumeration of spores surviving the disinfection treatment.

Organisms surviving a disinfection treatment are more exacting in their nutritive requirements. Also many spores in a population will not germinate in usual media but require the addition of special substances which act as stimulants to spore germination (Olsen & Scott, 1946; Schmidt, 1955; Hachisuka et al., 1955, 1956; Franklin & Clegg, 1956; Williams et al., 1957a, b; Amaha & Ordal, 1957; Woese et al., 1958; Jacobs & Harris, 1960).



Among the substances most commonly added to media for the estimation of spores surviving a killing influence are: soluble starch (e.g. Williams et al., 1957a), soluble starch plus skim milk (e.g. Franklin & Clegg, 1956), amino-acids particularly L-asparagine and amino-acids plus caramelized glucose (e.g. Hachisuka et al., 1955). Many workers have also used extended incubation periods for the accurate estimation of surviving spores (e.g. Franklin & Clegg, 1956).

It was decided to compare 3 media for enumeration of viable spores. These media were: (A) starch milk agar (SMA), i.e. nutrient agar containing 1% separated milk and 0.1% soluble starch; (B) nutrient agar containing 0.1% soluble starch, and (C) nutrient agar containing 0.5% L-asparagine and 1% glucose, the asparagine and glucose being autoclaved together as suggested by Hachisuka et al., (1955).

#### The effect of the medium and the period of incubation on the number of viable spores detected.

An estimate of the spore concentration was made by a direct microscopic examination. From this, the concentration of the spore suspension appeared to be approximately  $2 \times 10^8$ /ml. And so the  $10^{-4}$  to  $10^{-8}$  dilutions of the suspension in sterile distilled water were plated out using the three media, A, B and C. The same plates were counted after 3 days<sup>1</sup> and 7 days<sup>1</sup> incubation at 37°C.





Only counts of 30 - 500 colonies/standard Petri dish were used in the calculation of the number of viable organisms. This range was suggested by Wilson (1935). Counts smaller than 30 colonies/plate are subject to error caused by contamination, whereas counts greater than 500 colonies/plate are subject to errors from overcrowding.

It was found that the plate receiving 1 ml of the  $10^{-5}$  dilution was the one in the countable range of 30 - 500 colonies in all cases. The results are shown in Table 19 (a). After 7 days<sup>†</sup> incubation the agar in the plates was dried and cracked.

From this limited trial there appears to be no significant difference between either of the treatments (i.e. types of media and period of incubation).

The effect of heating in steam cabinet (approx. 200°F) for 15 min and the medium on the number of viable spores detected.

A sub-lethal heat-treatment has been reported by many workers to have a stimulatory effect on spore germination (e.g. Schmidt, (1955)).

A  $10^{-1}$  dilution of the spore suspension in sterile distilled water was heated in a steam cabinet for 15 min. This was then diluted serially in 9 ml of distilled water blanks, and a similar set of dilutions was made of the unheated spores. The  $10^{-5}$  to  $10^{-7}$  dilutions were plated using the three media, A, B and C.





The resultant counts after 3 days<sup>1</sup> incubation at 37°C are shown in Table 19 (b). The results show that spores of this particular strain of B. subtilis are not very resistant to heat and this particular heat treatment did not have the expected stimulatory effect on germination. Again there is no appreciable difference between the three media. It was accordingly decided to try a heat treatment less severe.

The effect of heating to 80°C for 10 min and the medium on the number of viable spores detected.

Dilutions of  $10^{-5}$ ,  $10^{-6}$  and  $10^{-7}$  of the spore suspension were plated out using the three media, and the dilutions were then heated in a water-bath at 80°C for 10 min and plated also using the three media. The results obtained are shown in Table 19 (c).

This heat treatment had no apparent stimulatory effect and again there was no appreciable difference between the three media.

It was thought possible that, although there was no significant difference between the counts obtained on the different media in these experiments, there might be some difference between the media in the detection of spores surviving treatment with sodium hydroxide. And so further experiments were made comparing the three media.



Comparison of the three media for the enumeration of spores  
surviving treatment with 3% sodium hydroxide at 100°F.

One ml of spore suspension was added to 100 ml of 3% sodium hydroxide solution at 100°F in an Erlenmeyer flask kept in a water-bath maintained at this temperature. After thorough mixing, a 10 ml sample was removed and added to 10 ml of hydrochloric acid of the same normality as the alkali and 10 ml of sterile phosphate buffer at pH approximately 7 containing a few drops of bromthymol blue indicator. (The buffer stock solution was prepared by dissolving 17 g of potassium dihydrogen phosphate in 250 ml distilled water and adjusting the pH to 7.2 with N sodium hydroxide. This was then made up to 500 ml with distilled water. A 10% solution of this stock solution in distilled water was used as the buffer). This neutralized solution was shaken thoroughly to mix and then diluted and plated using the three media. A sample was taken from the flask in a similar manner after the flask had been kept at 100°F for 12 hr. The results are shown in Table 19 (d).

There appears to be no difference in the count obtained on different media even after exposure of the spores to the action of 3% sodium hydroxide solution for 12 hr at 100°F.



Table 19. The effect of the medium in conjunction with (a) period of incubation, (b) heating the spore suspension at approx. 200°F (93°C) for 15 min, (c) heating the spore suspension at 80°C for 10 min, and (d) treating the spore suspension with 3% NaOH at 100°F for 12 hr, on the number of viable spores detected

Treatment	Colony counts calculated as the number of surviving (or viable) spores detected/ml of original spore suspension, on medium		
	A	B	C
(a) 3 days <sup>1</sup> incubation	1.2 x 10 <sup>7</sup>	1.5 x 10 <sup>7</sup>	1.4 x 10 <sup>7</sup>
7 days <sup>1</sup> incubation	1.2 x 10 <sup>7</sup>	1.6 x 10 <sup>7</sup>	1.4 x 10 <sup>7</sup>
(b) Unheated	1.8 x 10 <sup>7</sup>	1.9 x 10 <sup>7</sup>	2.3 x 10 <sup>7</sup>
Heated 93°C, 15 min	6.0 x 10 <sup>5</sup> #	1.0 x 10 <sup>5</sup> #	5.0 x 10 <sup>5</sup> #
(c) Unheated	1.4 x 10 <sup>7</sup>	1.4 x 10 <sup>7</sup>	1.4 x 10 <sup>7</sup>
Heated 80°C, 10 min	1.4 x 10 <sup>7</sup>	1.2 x 10 <sup>7</sup>	1.4 x 10 <sup>7</sup>
(d) 3% NaOH, 100°F, 0 hr	8.4 x 10 <sup>6</sup>	1.0 x 10 <sup>7</sup>	1.1 x 10 <sup>7</sup>
3% NaOH, 100°F, 12 hr	4.0 x 10 <sup>4</sup>	4.2 x 10 <sup>4</sup>	6.0 x 10 <sup>4</sup>

# colony count/plate too low to be sufficiently accurate.

$\cup T = \bigcup_{i=1}^n T_i$

- (1)  $T_1, T_2, \dots, T_n$  are disjoint
- (2)  $T_1, T_2, \dots, T_n$  are disjoint
- (3)  $T_1, T_2, \dots, T_n$  are disjoint
- (4)  $T_1, T_2, \dots, T_n$  are disjoint

$(T_1, T_2, \dots, T_n)$  is a partition of  $T$

$(T_1, T_2, \dots, T_n)$  is a partition of  $T$



Destruction of *B. subtilis* spores with 1.5%, 2%, 3% and 5%  
sodium hydroxide solutions at 60°F.

The same technique was used in this experiment as in the previous ones and this technique is similar to that used by Williams et al. (1957a, b), Franklin & Clegg (1956), Levine et al. (1927), etc. One ml of spore suspension was added to 100 ml of each of the concentrations of sodium hydroxide contained in Erlenmeyer flasks stoppered with cotton wool. The solutions were placed in a water-bath at least 12 hr before being inoculated to ensure that the correct temperature of the solution had been reached and for convenience. Then, at intervals, 10 ml samples were removed after thorough mixing of the contents of the flask, and added to tubes containing 10 ml of acid the same normality as the alkali and 10 ml of phosphate buffer at approximately pH 7. After thorough mixing by shaking, dilutions were made in distilled water and these plated out following a standard technique. All flasks and tubes were shaken thoroughly after each addition and before each removal of a sample. The three media were again compared.

1.5%, 2%, 3% and 5% sodium hydroxide solutions were used at 60°F and samples were taken at 0, 3 and 12 hr.

One ml of spore suspension was added to 100 ml of sterile distilled water in a sterile Erlenmeyer flask at 60°F. A 10 ml sample was removed and added to a sterile tube containing 10 ml sterile distilled water plus 10 ml sterile buffer. This, after shaking, was diluted and plated using the three media, in the same way as were the treatments. This served as a control.



The detailed results obtained are given in Table 20 and an analysis of the data given in Table 21.

Medium C gave counts generally slightly higher than A, while A gave counts slightly higher than B, though the differences probably are not significant.

It was decided that there was very little difference between the colony counts on the different media. If anything, medium A (SMA) and medium C (nutrient agar plus asparagine and glucose) proved slightly superior to medium B (nutrient agar plus soluble starch). And so for convenience and cheapness it was decided to use SMA as the medium for estimating the counts of viable spores in further experiments.



Table 20. Number of spores surviving treatment with different concentrations of sodium hydroxide at 60°F for different periods of time and using three media

Medium	Time of treatment (hr)	Organisms surviving treatment with 1.5% NaOH					Organisms surviving treatment with 2% NaOH				
		Actual counts in amounts of (ml)			Counts/ml of original suspension		Actual counts in amounts of (ml)			Counts/ml of original suspension	
		1	0.1	0.01	0.001	suspension	1	0.1	0.01	0.001	suspension
A	0	-	-	386	44	$1.3 \times 10^7$	-	-	328	50	$1.5 \times 10^7$
	3	-	780	89	-	$2.7 \times 10^6$	-	270	43	-	$1.0 \times 10^6$
	12	-	350	48	-	$1.4 \times 10^6$	-	304	35	-	$1.0 \times 10^6$
B	0	-	-	344	46	$1.4 \times 10^7$	-	-	354	48	$1.4 \times 10^7$
	3	-	460	41	-	$1.2 \times 10^6$	-	410	51	-	$1.6 \times 10^6$
	12	-	274	40	-	$1.0 \times 10^6$	-	204	31	-	$7.8 \times 10^5$
C	0	-	-	401	47	$1.4 \times 10^7$	-	-	470	38	$1.1 \times 10^7$
	3	-	-	210	-	$6.3 \times 10^6$	-	-	118	-	$3.5 \times 10^6$
	12	-	560	73	-	$2.2 \times 10^6$	-	451	53	-	$1.6 \times 10^6$
		Organisms surviving treatment with 3% NaOH					Organisms surviving treatment with 5% NaOH				
		Actual counts in amounts of (ml)			Counts/ml of original suspension		Actual counts in amounts of (ml)			Counts/ml of original suspension	
		1	0.1	0.01	0.001	suspension	1	0.1	0.01	0.001	suspension
A	0	-	-	375	44	$1.3 \times 10^7$	-	-	320	46	$1.4 \times 10^7$
	3	-	360	53	-	$1.6 \times 10^6$	-	241	15	-	$7.2 \times 10^5$
	12	-	240	20	-	$6.6 \times 10^5$	272	9	0	-	$8.1 \times 10^4$
B	0	-	-	307	25	$7.5 \times 10^6$	-	-	312	27	$8.1 \times 10^6$
	3	-	336	37	-	$1.1 \times 10^6$	-	198	17	-	$5.9 \times 10^5$
	12	-	102	12	-	$3.3 \times 10^5$	121	4	0	-	$3.6 \times 10^4$
C	0	-	-	387	44	$1.3 \times 10^7$	-	-	367	45	$1.4 \times 10^7$
	3	-	-	111	-	$3.3 \times 10^6$	-	355	44	-	$1.3 \times 10^6$
	12	-	181	17	-	$5.3 \times 10^5$	45	3	1	-	$1.4 \times 10^4$



Table 21. Comparison of media A, B and C for estimating the number of viable spores detected after treatment with different concentrations of sodium hydroxide solutions at 60°F for different periods of time

Concn NaOH (%)	Time of treatment (hr)	Counts x 10 <sup>5</sup> /ml of original spore suspension on medium		
		A	B	C
1.5	0	132	138	141
	3	26.7	12.3	63
	12	14.4	10.1	21.9
2	0	150	144	114
	3	10.5	15.9	35.4
	12	10.5	7.8	15.9
3	0	132	75	132
	3	15.9	11.1	33.3
	12	6.6	3.3	5.3
5	0	138	81	135
	3	7.2	5.9	13.2
	12	0.81	0.36	0.14





B. subtilis SM 761 was the organism chosen for study as its spores are even more resistant than those used in the preliminary studies, and this organism was the one used by Franklin & Clegg (1956) in similar studies.

A spore suspension was prepared as before. One ml of a 24-hr broth culture was inoculated into each of 16 Roux bottles containing the sporulating medium. After 7 days<sup>†</sup> incubation at 37°C, the growth was loosened with a sterile glass rod and washed off with sterile distilled water. This crude suspension was shaken with glass beads for 3-1/2 hr and then centrifuged at 1,000 revolutions/min and the sediment discarded. The supernatant was heated in the steamer (about 200°F) for 15 - 20 min.

The suspension was washed with sterile distilled water 5 times, centrifuging at 3,000 revolutions/min for 45 min to bring down the spores and the suspension stored in the refrigerator in a stoppered bottle until required.

Disinfection experiment at 60°F using 0%, 1.5%, 2%, 3% and 5% sodium hydroxide for 0, 3, 6, 9 and 12 hr.

The standard procedure was again used in this experiment. Each of the 5 flasks containing 100 ml of the sterile solution was inoculated with 1 ml of the thoroughly shaken spore suspension. As soon as the spore suspension and solution had been thoroughly mixed by shaking, a 10 ml aliquot was removed, neutralized, diluted and plated in triplicate



using SMA. Two drops of bromthymol blue indicator were added to the solution to check the pH. It was thought that this indicator would probably be sterile or near-sterile but to check this the indicator was plated out. It was found that the indicator had a plate count of approximately 600 colonies/drop with approximately 10 mould colonies/drop.

The plates of the control treatment (i.e. using distilled water instead of sodium hydroxide) showed heavy contamination, but when these plates were counted, only colonies resembling B. subtilis colonies were counted, though this selection was difficult as this organism produces many forms of colony. It is possible also that the indicator contributed some of the B. subtilis organisms or organisms producing colonies resembling those of this organism. The indicator was added to the tubes of acid and buffer several hours before these were neutralized. Plates from these solutions showed little contamination and it is presumed that the low pH destroyed most of the organisms except mould and bacterial spores.

For other experiments therefore, a suitable quantity of bromthymol blue indicator was added to the buffer solution before this was sterilized.

Three dilutions were plated out for each sample in order to cover the dilution which would be in the countable range of 30 - 500 colonies/plate. The arithmetic mean of the three replicates was found for each dilution, and where two consecutive dilutions were in the range of 30 - 500, the arithmetic mean of the two estimations was used.

The results are shown in Table 22.



Table 22. The effect of time of treatment with different concentrations of sodium hydroxide on the number of spores surviving

Concn NaOH (%)	Number of spores/ml of original suspension surviving treatment				
	Time of treatment (hr)				
	0	3	6	9	12
0	$9.8 \times 10^6$	$9.2 \times 10^6$	$9.2 \times 10^6$	$5.1 \times 10^6$	$2.0 \times 10^7$
1.5	$4.3 \times 10^7$	$1.8 \times 10^6$	$7.0 \times 10^5$	$2.6 \times 10^6$	$1.0 \times 10^6$
2	$2.0 \times 10^7$	$8.6 \times 10^5$	$6.0 \times 10^5$	$4.4 \times 10^5$	$2.9 \times 10^5$
3	$9.8 \times 10^6$	$7.6 \times 10^5$	$9.4 \times 10^4$	$9.0 \times 10^4$	$2.2 \times 10^4$
5	$9.8 \times 10^6$	$5.9 \times 10^5$	$1.3 \times 10^4$	$<1 \times 10^2$	$<1 \times 10^2$

These data are shown in Fig. 8, where the logarithm of the count of viable organisms/ml of original spore suspension is plotted against time. From this graph it can be seen that the points are scattered and do not follow a smooth curve. Using the technique as it is, it is possible to detect accurately only a 99.9% kill. If the initial spore concentration is  $1 \times 10^7$ /ml, and the lowest concentration of organisms accurately detected is  $9 \times 10^3$ /ml (i.e. 30 colonies/plate of the undiluted neutralized solution), the greatest percentage kill detectable is:

$$10,000,000 - 9,000 = 9,991,000$$

$$\frac{9,991,000}{10,000,000} \times 100 = 99.9\% \text{ kill}$$









Fig. 8. The effect of different concentrations of sodium hydroxide solutions on *B. subtilis* spores at 60°F. Open circles, 0% NaOH; open squares, 1.5% NaOH; open triangles, 2% NaOH; full circles, 3% NaOH; full squares, 5% NaOH.



It was realized that large numbers of Petri dishes, as would be required for the final experiments would be unwieldy, and so it was decided to use a roll tube apparatus for making the counts. This method requires less incubator space, is easier and quicker to use and is generally more convenient.

Disinfection experiment at 50°F using 0%, 1.5%, 2%, 3% and 5% sodium hydroxide solutions for periods up to 24 hr

The same technique was employed in this experiment as in the previous ones except that roll-tubes were used instead of Petri dishes. Duplicate roll-tubes were used for each dilution and 0.5 ml of the inoculum was added to each tube.

The counts for replicate roll-tubes were summed. Since the area of the agar surface in a roll-tube is approximately 37 sq cm (i.e. approximately half the surface area of a Petri dish) the accurately countable range per roll-tube was taken as approximately 15 - 250 colonies. Since only 0.5 ml of inoculum was used per tube, counts of 30 - 500/ml (occasionally counts up to 600/ml) were those used in estimating the viable count. Again if 2 consecutive dilutions gave counts within the range of 30 - 500/ml, the arithmetic mean of these two estimates was used.

The results are plotted in Fig. 9. From this point on, the data obtained are far too numerous to report in full, and so the data are given only in the form of graphs.





Fig. 9. The effect of different concentrations of sodium hydroxide solutions on *B. subtilis* spores at 50°F. Open circles, 0% NaOH; open squares, 1.5% NaOH; full squares, 2% NaOH; open triangles, 3% NaOH; full circles, 5% NaOH.



The points in Fig. 9 are considerably scattered. This suggested that greater replication was needed. Even the points for the control treatment were scattered. In further studies it was decided to use duplicate sets of dilutions in addition to duplicate roll-tubes for each dilution. Special attention was to be paid to thorough mixing of every suspension from which a transfer was made, by shaking vigorously by hand.

The points on the graph in Fig. 9 for time 0 hr vary considerably. It was thought that this variation might have been due partly to a sub-lethal stimulatory effect on spore germination by a brief contact with the lethal agent, and due partly to an uneven inoculation of the different solutions with the spore suspension. If the former explanation were correct, it would mean that the SMA does not stimulate all the spores to germinate.

For further work, to ensure a more even inoculation of the different solutions, it was decided to dilute 5 ml of the spore suspension with sterile distilled water, and, after thorough shaking, to inoculate each solution of sodium hydroxide with 10 ml of the diluted suspension. This required using 90 ml of sodium hydroxide solution of a concentration adjusted so that when 90 ml was diluted to 100 ml with 10 ml of the spore suspension in distilled water, the resultant concentration would be the concentration desired (i.e. 1.5%, 2%, 3% or 5%).

The normalities of the four solutions used with the corresponding normalities and concentrations when 90 ml is diluted to 100 ml with





distilled water are given in Table 23.

Table 23. Normalities and concentrations of sodium hydroxide solutions used for the treatment solutions

Normality of solution	Normality of solution when diluted 9:1 with distilled water		Concentration
0.417	0.375	=	1.5%
0.556	0.500	=	2%
0.833	0.750	=	3%
1.389	1.250	=	5%

Disinfection experiment at 50°F using 0%, 1.4%, 1.8%, 2.7% and 4.5% sodium hydroxide solutions for periods up to 24 hr.

A preliminary trial was made using the sodium hydroxide solutions at the concentrations used previously (i.e. 1.5%, 2%, 3% and 5%) but using the new technique of inoculating 90 ml of solution with 10 ml of the diluted spore suspension. The resultant normalities of the four solutions, 1.5%, 2%, 3% and 5% were 0.342N (1.4%), 0.457 N (1.8%), 0.682N (2.7%) and 1.130N (4.5%) respectively. Nine ml of acid was used instead of 10 ml to neutralize the 10 ml samples of alkali making the total volume of the neutralized sample 29 ml instead of 30 ml.



Attention was paid to more thorough mixing of the suspensions and dilutions, and glass beads were added to the test solutions to ensure more thorough mixing when the flasks were shaken. Duplicate dilutions in addition to duplicate roll-tubes were used, so that a count for each dilution was based on 4 individual counts. If 2 consecutive dilutions were in the countable range, the final count was based on 8 individual counts.

The results are given in Fig. 10. It can be seen that points on the graph are much less scattered than those obtained previously, indicating that more attention to mixing each suspension and the use of replicate series of dilutions are necessary to produce satisfactory results. The points at 0 hr are still somewhat scattered, the counts for the sodium hydroxide solutions being higher than that of the control treatment. This again suggests that a treatment for a short period of time with the sodium hydroxide solutions stimulates spore germination and that without this stimulation, not all of the spores germinate. Lower concentrations of sodium hydroxide produce a higher initial count, the initial count for the 1.4% treatment being almost 100 times greater than that for the control treatment. More accurate standardization of the amount of inoculum added to the sodium hydroxide solutions does not appear to be a factor significantly affecting the initial count.

For all previous experiments using roll-tubes, 5 ml of medium was used per tube. This resulted in the layer of agar being too thick and in consequence crowded tubes were sometimes difficult to count, with the possibility that colonies might occasionally be counted twice





Fig. 10. The effect of different concentrations of sodium hydroxide solutions on *B. subtilis* spores at 50°F. Open circles, 0% NaOH; open squares, 1.4% NaOH; open triangles, 1.8% NaOH; full circles, 2.7% NaOH; full squares, 4.5% NaOH.





owing to error due to parallax when the tube was rotated. Accordingly it was decided to use approximately 3 - 4 ml of medium per tube to reduce the thickness of the agar layer.

Occasionally the agar column slipped and so the concentration of agar in the SMA medium was increased from 1.3% to 2%.

Disinfection experiment at 50°F using 0%, 1.5%, 2%, 3% and 5%  
sodium hydroxide solutions for periods up to 24 hr

The experiment at 50°F was repeated using the new concentrations of sodium hydroxide. The diluted spore suspension was placed in the water-bath along with the test solutions at least 12 hr before inoculation of the test solutions to ensure that the correct temperature had been attained and for convenience. Where it was expected that the count of viable organisms surviving would be low, 10 replicate roll-tubes were used, each being inoculated with 0.5 ml of the undiluted neutralized solution.

The results are plotted in Fig. 11. Though the same technique was used and the same precautions against inadequate mixing were taken as in the previous experiment, the points are considerably more scattered.

There was insufficient spore suspension for many more trials in this experiment and, as it seemed more desirable to use a more concentrated suspension, it was decided to make another suspension.





Fig. 11. The effect of different concentrations of sodium hydroxide solutions on *B. subtilis* spores at 50°F. Open circles, 0% NaOH; open squares, 1.5% NaOH; full squares, 2% NaOH; open triangles, 3% NaOH; full circles, 5% NaOH.



A freeze-dried culture of B. subtilis SM 761 was opened and an agar surface streaked with the culture. A representative colony was picked off and sub-cultured on nutrient agar slopes incubating at 37°C for 24 hr. The growth was washed off a slope with sterile distilled water and this suspension used to inoculate the surface of nutrient agar contained in 2 l-litre flasks. After incubation at 37°C for 44 hr, the growth was suspended in sterile distilled water and used to inoculate the sporulating medium.

The sporulating medium was the same as used before except that 10 ml of a solution containing 0.01% Mn was used per litre of medium. This gave a final concentration of Mn of 1 p/m. The range of Mn concentration suggested by Charney et al. (1951) to stimulate spore production was 0.1 - 10 p/m.

One ml of the suspension of B. subtilis was used to inoculate the surface of agar in each of approximately 150 Roux bottles. For convenience, the Roux bottles were kept in a warm room at 91 - 97°F for 7 days, the incubators available to be used at 37°C (98°F) having insufficient capacity to hold this quantity of Roux bottles.

The procedure for harvesting and washing the spores was the same as used previously. After shaking to break up clumps, the suspension was heated at 85°C for 5 min. The suspension was washed 5 times with sterile distilled water and the volume of the purified suspension was adjusted to 100 ml with sterile distilled water. This was then heated momentarily in the steamer, cooled and stored in the refrigerator.





A direct microscopic count using a Levy-Hausser counting chamber gave the concentration as approximately  $1 \times 10^{10}$  spores/ml. The volume of the suspension was adjusted to 150 ml with sterile distilled water giving a concentration of approximately  $7 \times 10^9$ /ml.

A sample of the suspension was plated out on SMA using roll-tubes. From this the concentration of spores was estimated at  $7.4 \times 10^7$ /ml, i.e. approximately 1% of the direct microscopic count.

In order to see whether a further sub-lethal heat treatment would have any stimulatory effect on the number of viable spores detected on SMA, the following experiment was conducted.

The effect of sub-lethal heat treatments on the number of spores forming colonies on SMA

A  $10^{-1}$  dilution of the spore suspension was heated in a boiling water-bath for 0, 10, 20, 30, 45, 60 and 90 min. The spore suspension was held in a test-tube marked at 1 ml intervals. There was some slight discrepancy in that the difference in volume of water at room temperature and at boiling point was ignored. If there was any loss of water due to evaporation, distilled water was added up to the appropriate level and the suspension mixed. One ml samples were removed, after mixing the suspension with a mechanical mixer (Vortex Jr mixer), at the above stated time intervals, diluted appropriately in sterile distilled water and plated out using SMA and using Petri dishes.

The results obtained are given in Table 24. From these data





Table 24.                      The effects of sub-lethal heat treatments  
on the number of spores detected on SMA

Time of heating (min)		Colony count			Calculated/ml of spore suspension
		/0.5ml of dilution			
		10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	
rep					
0	a	390	38	7	7.1 x 10 <sup>7</sup>
	b	330	32	8	
10	a	22	2	1	5.3 x 10 <sup>6</sup>
	b	31	3	1	
20	a	40	3	0	7.7 x 10 <sup>6</sup>
	b	36	3	0	
30	a	31	2	2	4.8 x 10 <sup>6</sup>
	b	17	9	0	
45	a	36	4	2	7.6 x 10 <sup>6</sup>
	b	40	9	1	
60	a	29	9	2	7.4 x 10 <sup>6</sup>
	b	45	4	3	
90	a	52	7	0	1.0 x 10 <sup>7</sup>
	b	52	8	0	

it was concluded that there was no significant stimulatory effect by the heat treatments given. The colony count appears to be reduced 10-fold by heating in a boiling water-bath for 10 min. It was decided not to subject the suspension to a further heat treatment.



### Final experiments

Trial experiments were carried out at 37°F, 50°F and 82°F in order to estimate the dilutions to use for the different treatments of temperature, concentration of sodium hydroxide and time in the final experiments. Particular care was taken to see that every flask and tube was shaken thoroughly before a sample was withdrawn. Test-tubes were shaken with the aid of a Vortex Jr mechanical mixer and flasks of treatment solution containing glass beads were rotated vigorously approximately 40 times in each direction (i.e. clockwise and anti-clockwise) before sampling. The neutralizing solutions were contained in large medical flat bottles and these were shaken well after inoculation and before sampling. The flasks were stoppered with cotton wool plugs and covered with aluminium foil to reduce evaporation since treatment periods of up to 100 hr were used in some cases.

The results of the trials at 37°F, 50°F and 82°F are plotted in Figs. 12, 13 and 14. For purposes of predicting the correct dilutions to use at different temperatures and concentrations of sodium hydroxide, the viable counts for the different concentrations of sodium hydroxide were plotted against temperature, for treatment times of 3, 6, 12 and 24 hr. These graphs are shown in Fig. 15 where curves are drawn through the points obtained. In this way it was possible to predict approximately the concentration of viable organisms at any temperature, concentration of sodium hydroxide and time in the range being studied.



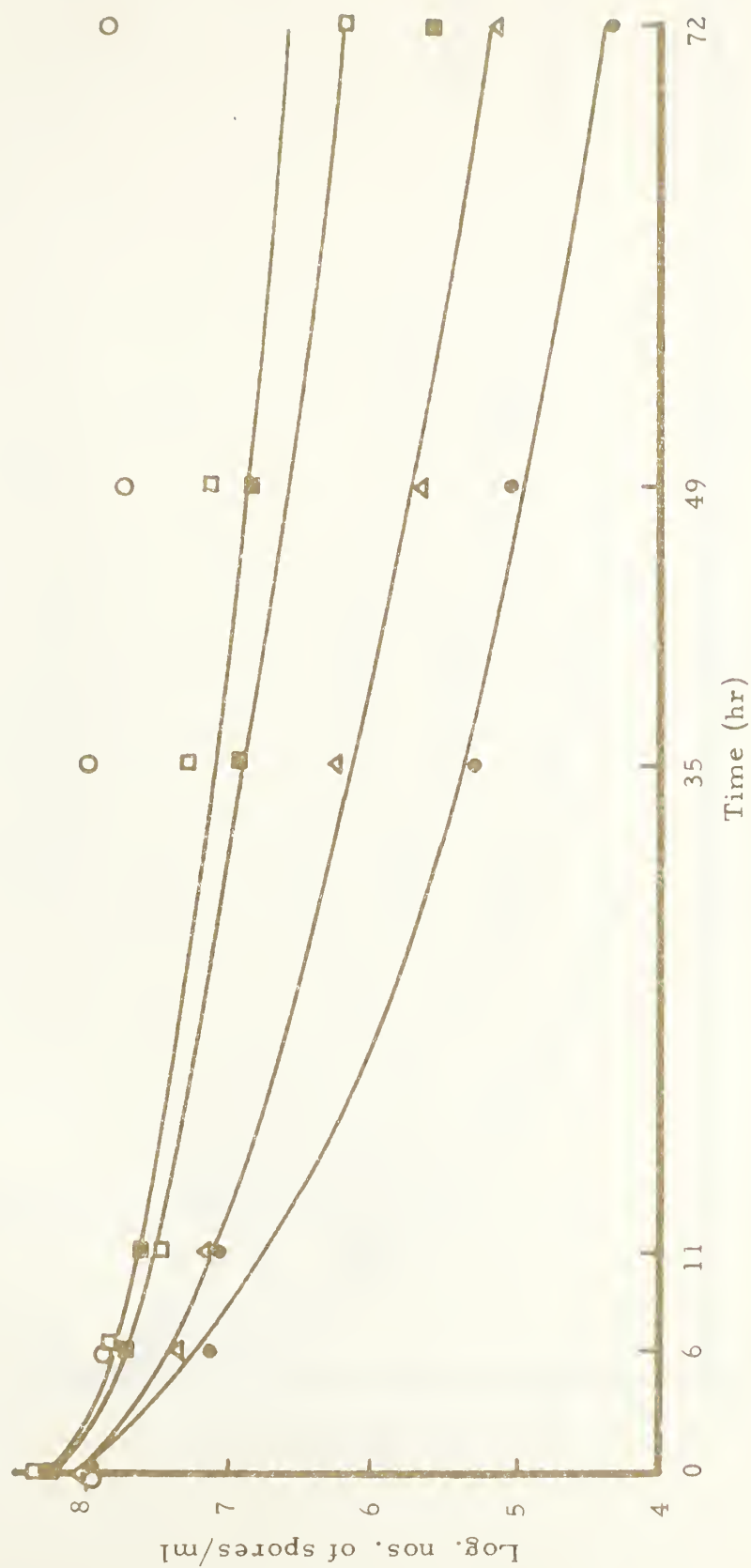


Fig. 12. The effect of different concentrations of sodium hydroxide solutions on *B. subtilis* spores at 37°F. Open circles, 0% NaOH; open squares, 1.5% NaOH; full squares, 2% NaOH; open triangles, 3% NaOH; full circles, 5% NaOH.







Fig. 13. The effect of different concentrations of sodium hydroxide solutions on *B. subtilis* spores at 50°F. Open circles, 0% NaOH; open squares, 1.5% NaOH; full squares, 2% NaOH; open triangles, 3% NaOH; full circles, 5% NaOH.



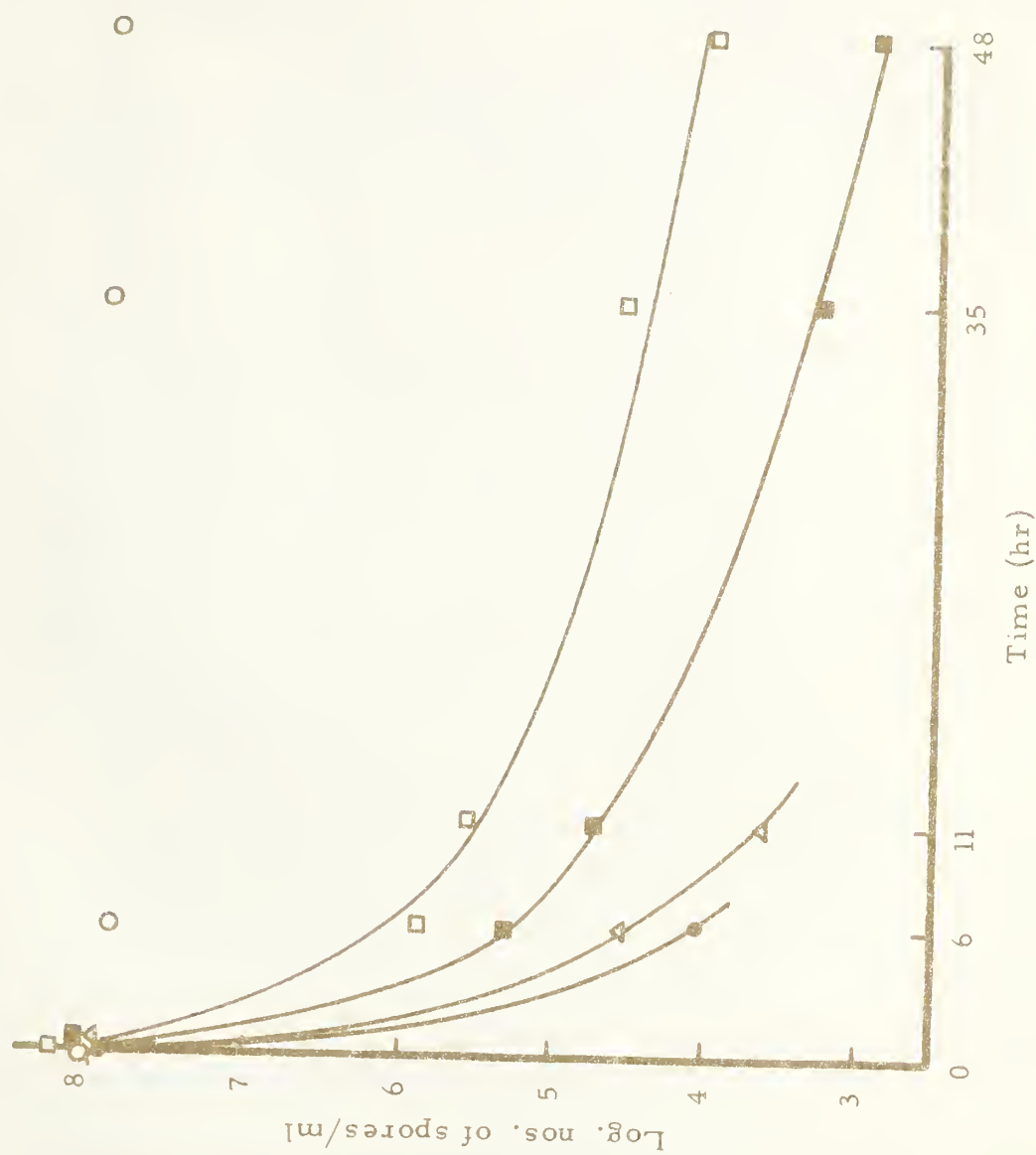


Fig. 14.

The effect of different concentrations of sodium hydroxide solutions on *B. subtilis* spores at 82°F. Open circles, 0% NaOH; open squares, 1.5% NaOH; full squares, 2% NaOH; open triangles, 3% NaOH; full circles, 5% NaOH.



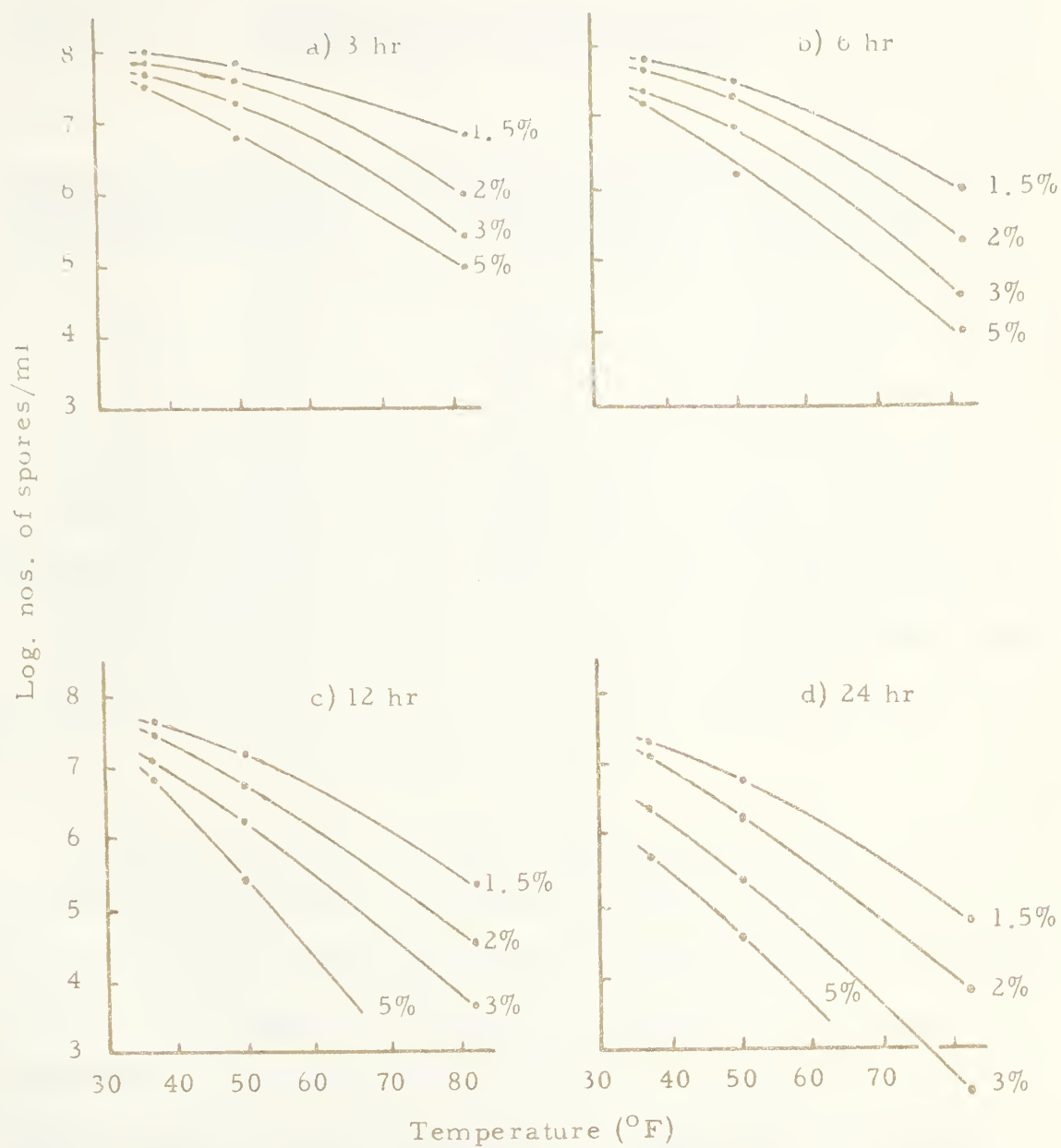


Fig. 15. Number of spores of *B. subtilis* surviving treatment with different concentrations of sodium hydroxide solutions for different treatment times.



Table 25 was constructed to indicate which dilutions were to be used.

Table 25. Dilutions to be used for predicted concentrations of viable spores

Predicted concentration of viable spores/ml	Principle dilution to be used	Range of dilutions to be used
$1.5 \times 10^8 - 1.5 \times 10^9$	$10^{-4}$	$10^{-3}$ , $10^{-4}$ , $10^{-5}$
$1.5 \times 10^7 - 1.5 \times 10^8$	$10^{-3}$	$10^{-2}$ , $10^{-3}$ , $10^{-4}$
$1.5 \times 10^6 - 1.5 \times 10^7$	$10^{-2}$	$10^{-1}$ , $10^{-2}$ , $10^{-3}$
$1.5 \times 10^5 - 1.5 \times 10^6$	$10^{-1}$	$10^0$ , $10^{-1}$ , $10^{-2}$
$1.5 \times 10^4 - 1.5 \times 10^5$	$10^0$	$6 \times 10^0$ , $6 \times 10^{-1}$
$1.5 \times 10^3 - 1.5 \times 10^4$		$10 \times 10^0$ , 10ml Roux
Less than $1.5 \times 10^3$		2 x 10ml Roux

Where the count/ml was high, dilutions either side of the one estimated to be the correct one were also used to ensure that the range was covered sufficiently in case the estimate was not sufficiently accurate, i.e. 3 consecutive dilutions were used for each sample. For smaller concentrations 10 roll-tubes of the undiluted neutralized suspension were used and also 10 ml of the neutralized solution was added to SMA contained in a Roux bottle. Where the concentration was even smaller, 2 Roux bottles were used.





The final experiments were carried out at 34°, 40°, 50°, 60° and 70°F. Control treatments using distilled water instead of sodium hydroxide solution were included in each experiment except the one at 34°F. It was hoped to use an incubation temperature of 30°F but this temperature proved difficult to obtain with the water-bath used. It was because it was intended to use 30°F that the control treatment with distilled water was omitted for this experiment.

The results of these experiments are shown in Figs. 16, 17, 18, 19 and 20, where the logarithms of the number of surviving organisms/ml of original spore suspension are plotted against time for each concentration of sodium hydroxide. Smooth curves are drawn through the points as accurately as possible. The same time-log. survivor curves are shown in Figs. 21, 22, 23 and 24 where the curves at different temperatures for one concentration of sodium hydroxide only are drawn on the same graph.

The concentration of the spore suspension was approximately  $1.0 \times 10^8$ /ml. When  $1.0 \times 10^6$  spores/ml were still viable, the percentage kill was 99%. The times to produce 99% destruction (i.e. the times at which the curves cross the  $1.0 \times 10^6$  abscissa on the graphs) for different concentrations of sodium hydroxide solution and at different temperatures are given in Table 26. These data are plotted in the form of a 3-dimensional graph in Fig. 25.

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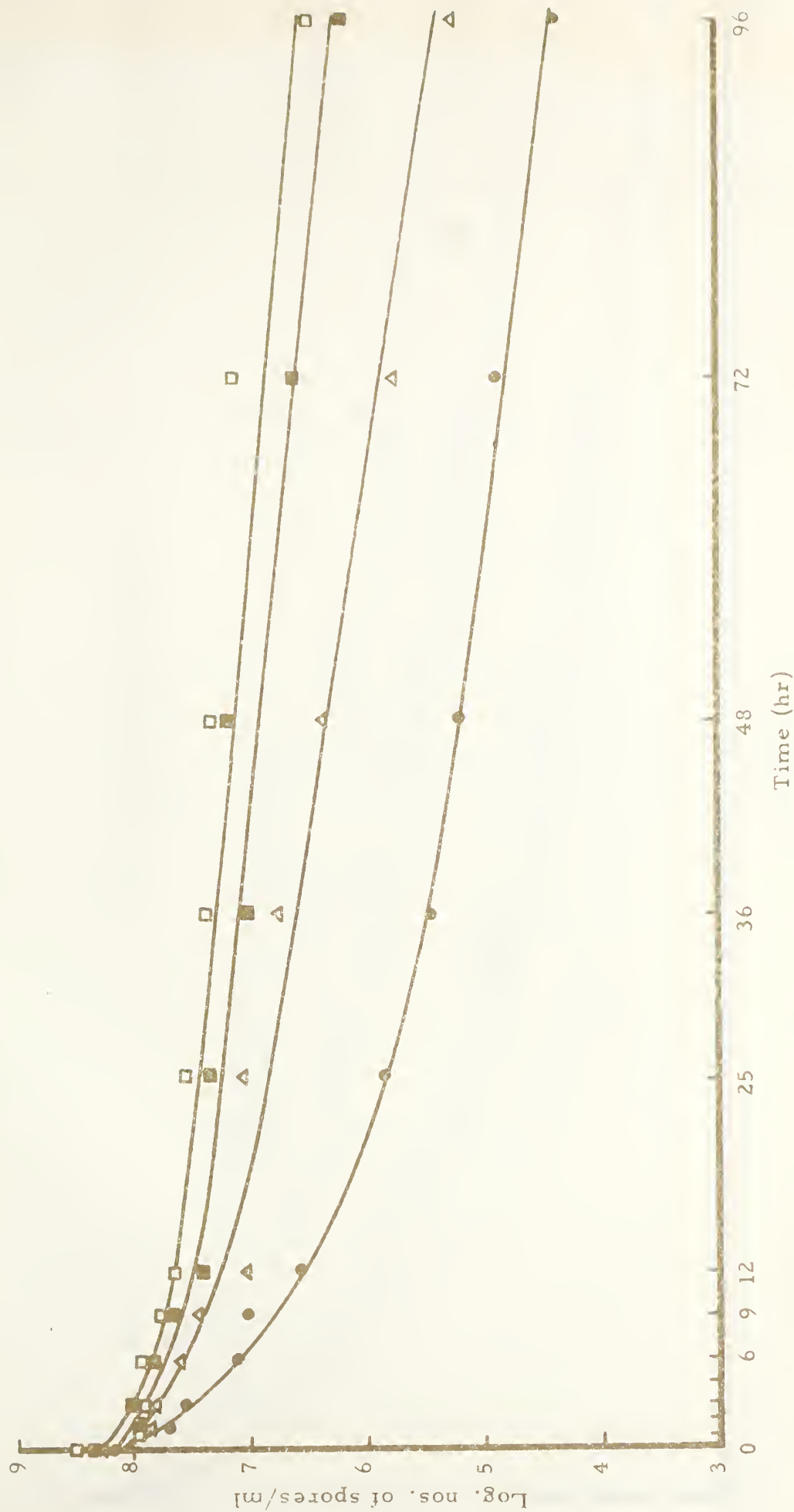


Fig. 16. The effect of different concentrations of sodium hydroxide solutions on *B. subtilis* spores at 34°F. Open squares, 1.5% NaOH; full squares, 2% NaOH; open triangles, 3% NaOH; full circles, 5% NaOH.





Fig. 17. The effect of different concentrations of sodium hydroxide solutions on *B. subtilis* spores at 40°F. Open circles, 0% NaOH; open squares, 1.5% NaOH; full squares, 2% NaOH; open triangles, 3% NaOH; full circles, 5% NaOH.





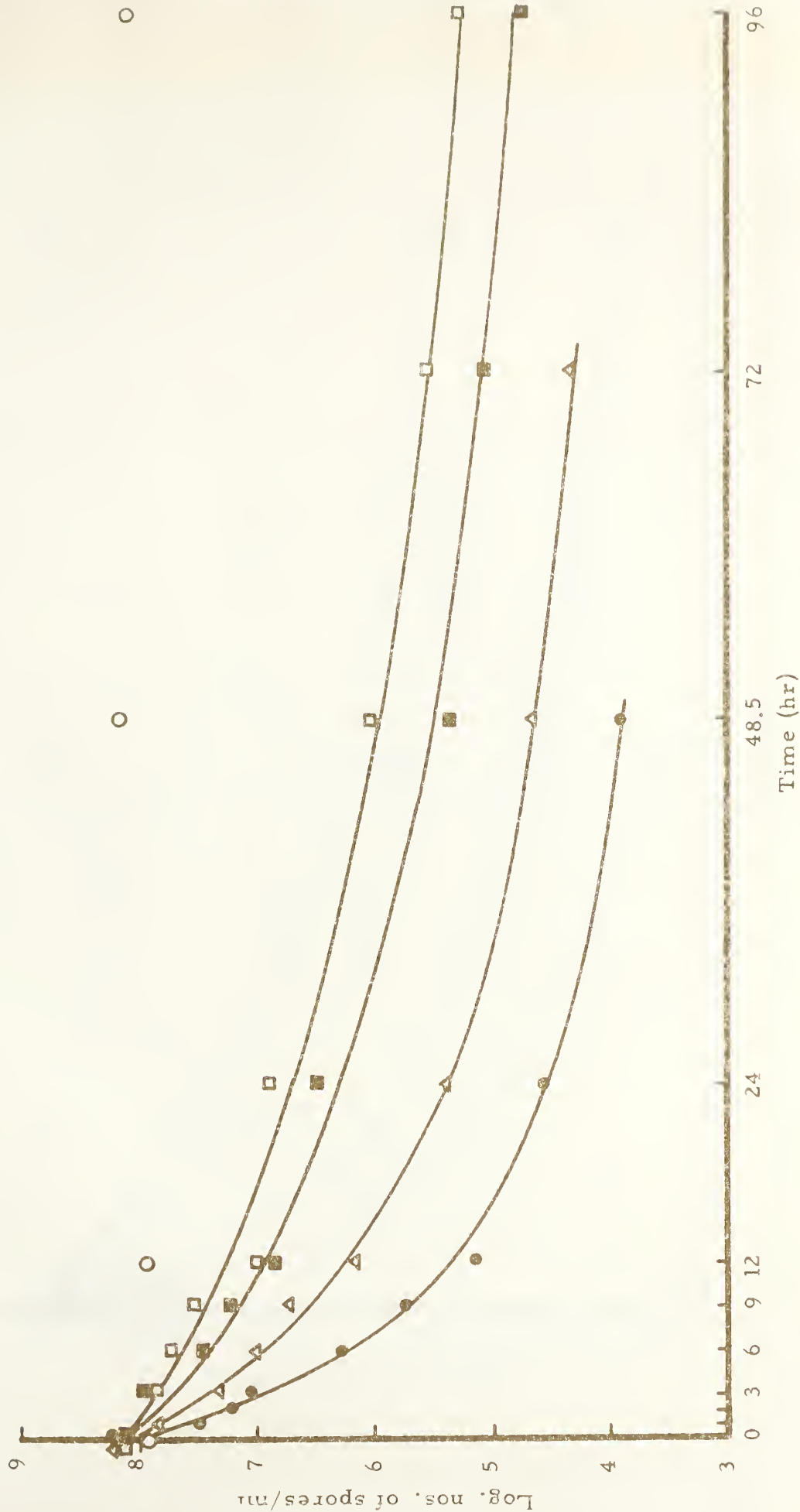


Fig. 18. The effect of different concentrations of sodium hydroxide solutions on *B. subtilis* spores at 50°F. Open circles, 0% NaOH; open squares, 1.5% NaOH; Full squares, 2% NaOH; open triangles, 3% NaOH; full circles, 5% NaOH.



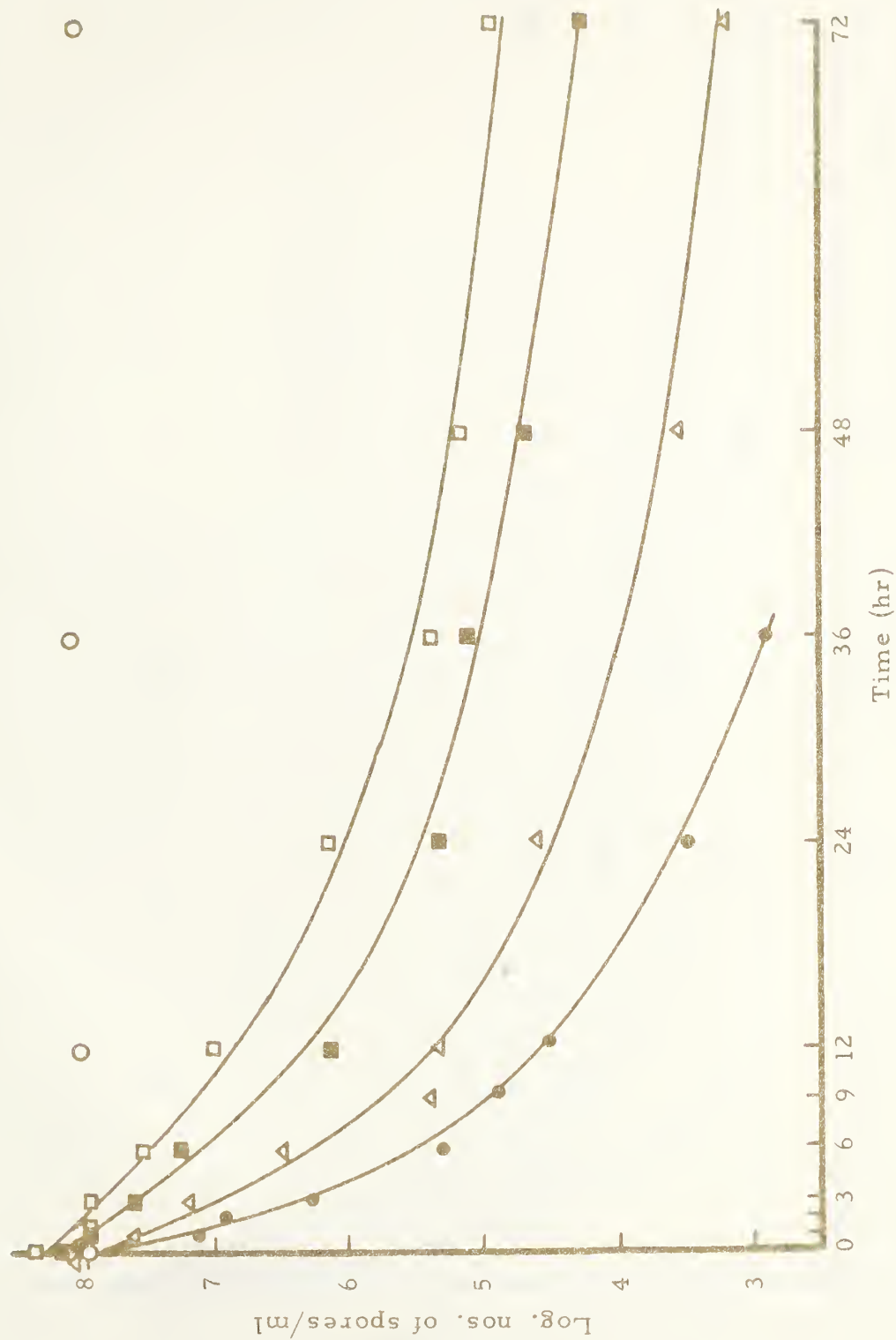


Fig. 19. The effect of different concentrations of sodium hydroxide solutions on *B. subtilis* spores at 60°F. Open circles, 0% NaOH; open squares, 1.5% NaOH; open triangles, 3% NaOH; full circles, 5% NaOH.





Fig. 20. The effect of different concentrations of sodium hydroxide solutions on *B. subtilis* spores at 70°F. Open circles, 0% NaOH; open squares, 1.5% NaOH; full squares, 2% NaOH; open triangles, 3% NaOH; full circles, 5% NaOH.





Fig. 21. The effect of temperature (°F) on disinfection of *B. subtilis* spores with 1.5% sodium hydroxide solution.





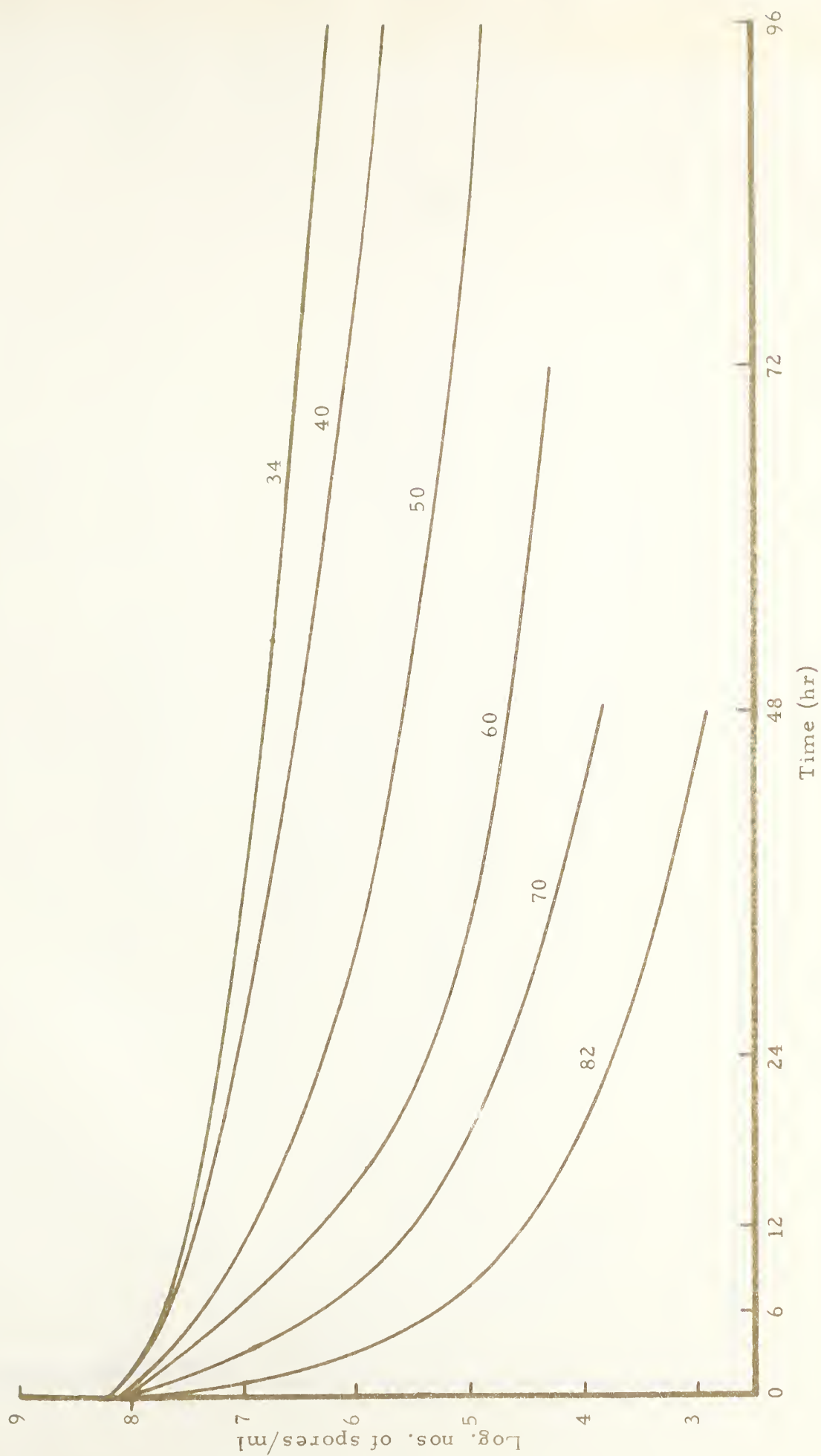


Fig. 22. The effect of temperature ( $^{\circ}\text{F}$ ) on disinfection of *B. subtilis* spores with 2% sodium hydroxide solution.



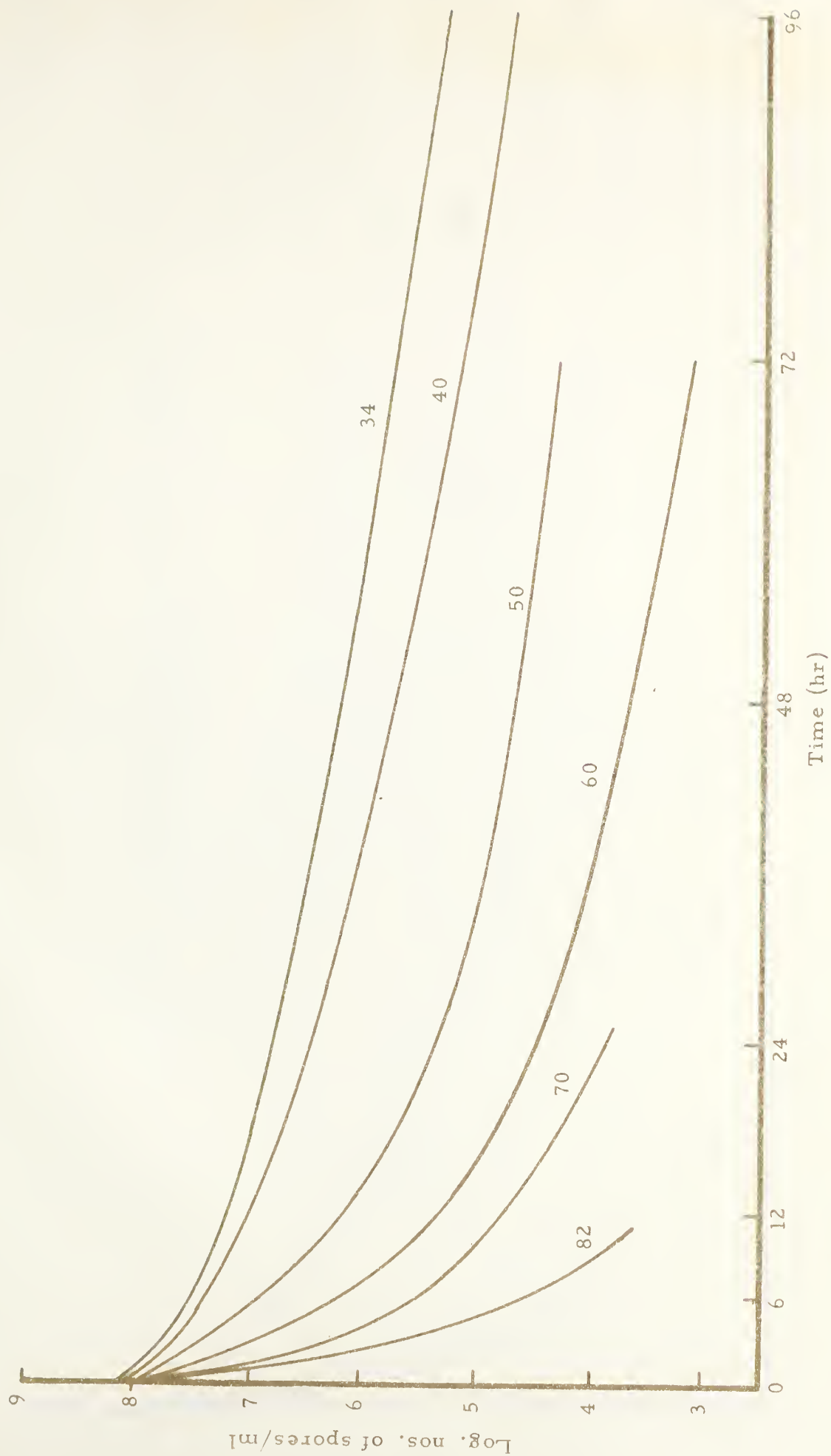


Fig. 23. The effect of temperature ( $^{\circ}\text{F}$ ) on disinfection of *B. subtilis* spores with 3% sodium hydroxide solution.



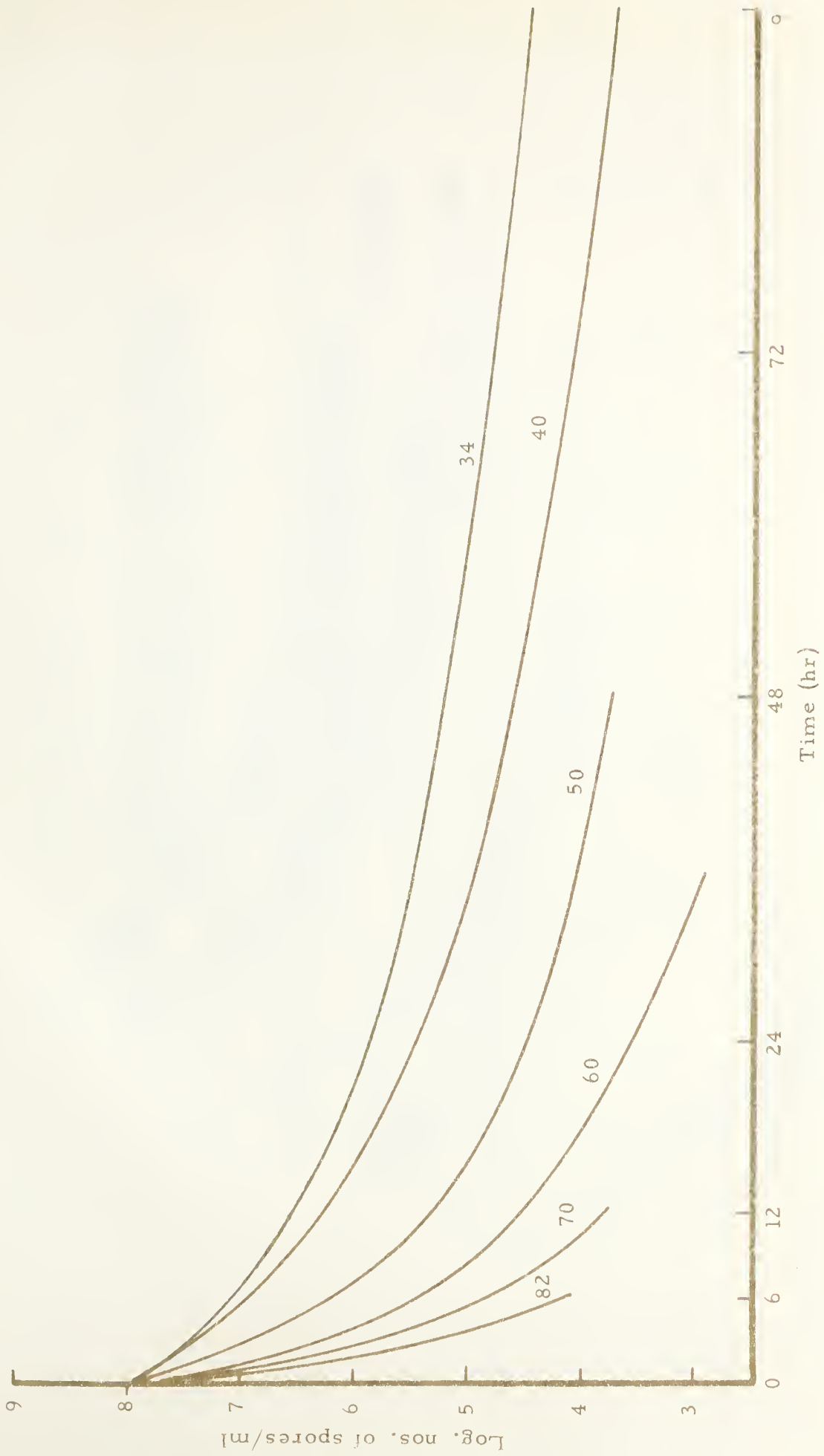


Fig. 24. The effect of temperature ( $^{\circ}\text{F}$ ) on disinfection of *B. subtilis* spores with 5% sodium hydroxide solution.





Table 26. The effect of concentration of sodium hydroxide solutions and temperature on the time required to produce 99% destruction of B. subtilis spores

Conc <sup>n</sup> NaOH (%)	Time of treatment (hr) required to produce 99% destruction of <u>B. subtilis</u> spores at				
	Temperature (°F)				
	34	40	50	60	82
1.5	150	120	49	26	13
2	120	76	32	16	8
3	62	44	15	5	3.2
5	24	16	7	4	2
					7.5
					3.2
					1.6
					1.5

Data pertaining to Fig. 25.



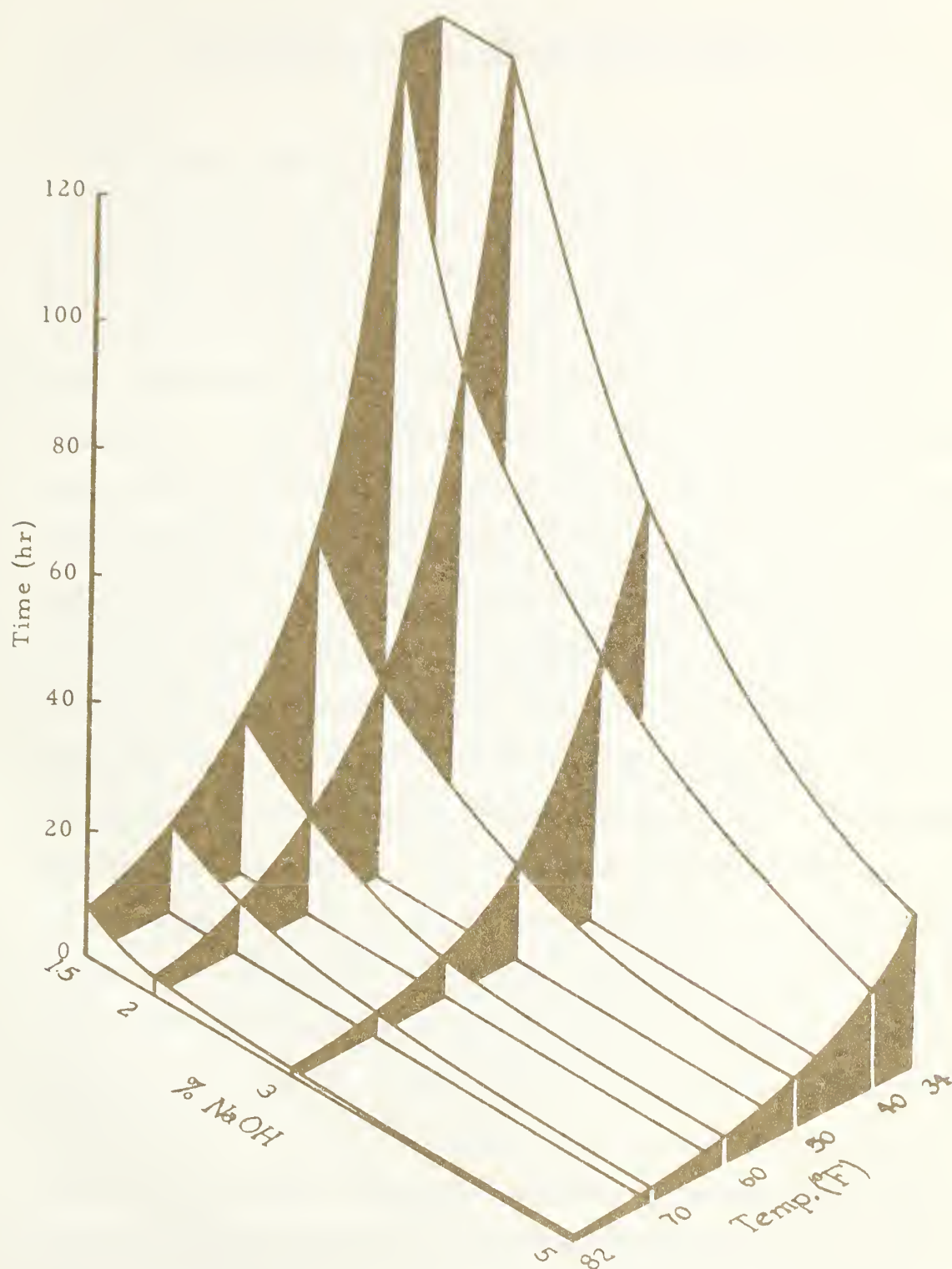


Fig. 25. The times required to produce 99% destruction of *B. subtilis* spores at different concentrations of sodium hydroxide and at different temperatures.



## DISCUSSION AND CONCLUSIONS OF PART II

The medium chosen (SMA) for enumeration of the organisms surviving treatment with sodium hydroxide solutions did not appear to stimulate germination of 100% of the spores of the first suspension of B. subtilis SM 761 (used in the preliminary experiments), which were not subjected to treatment with sodium hydroxide, i.e. those of the control. With this suspension the colony counts of spores treated with sodium hydroxide solution for 0 hr (i.e. momentarily) were considerably greater than those of the control, suggesting a stimulation of germination by a sub-lethal treatment with sodium hydroxide. With the second suspension of B. subtilis SM761 spores (the suspension used for the final experiments) this effect was less noticeable, i.e. while the counts at 0 hr for the sodium hydroxide treatments were greater than the counts for the control, the differences were less than with the first suspension. The only differences in the preparation of the two suspensions were: (1) the first suspension was heated to approximately 200°F (93°C) for 15 - 20 min; the second suspension was heated only to 85°C for 5 min, (2) for the production of the first suspension, a concentration of 0.01 p/m Mn was used, but for the second a concentration of 1 p/m, and (3) the temperatures at which the spores were grown. It is doubtful whether the different heat treatments given to the two suspensions would cause any difference between the suspensions since sub-lethal heat treatments of up to 90 min at 100°C were given to the second suspension without any effect. It is also doubtful whether





the slight difference in the temperature at which the spores were produced would have any effect. It is possible, however, that the different concentrations of manganese used in the sporulating medium contributed to the difference between the two suspensions since the composition of the medium used for the production of spores affects the resistance of the spores produced (e.g. Charney et al., 1951; Curran, 1952; Schmidt, 1955; Amaha & Ordal, 1957).

Even though the greatest care was taken in carrying out the experiments, there is considerable scattering of the points on the graphs particularly with the slower rates of disinfection. Using replicate dilutions in addition to replicate plates or roll-tubes and using a mechanical mixer to mix the suspensions, helped to reduce the variation to some extent but did not eliminate it entirely. It is presumed that much of the variation in the results arises from the way in which the organisms are dispersed in the suspensions, and thus the variation in results could be reduced by the use of an even greater number of replicates.

The curves obtained in the final experiments when the logarithms of the number of spores surviving are plotted against time are generally concave, i.e. disinfection commences at a rapid rate and then gradually and progressively slows down.

There is a direct relationship between both (i) overall rate of disinfection and concentration of sodium hydroxide solution in the range 1.5% to 5%, and (ii) overall rate of disinfection and temperature in the range 34°F to 82°F.





At the lower temperatures and concentrations of sodium hydroxide the rates of disinfection are very low. Even with the higher rates of disinfection, the rates begin to slow down markedly when there is still a considerable number of organisms remaining viable. This, at first glance, indicates that a relatively large proportion of the population is of great resistance. However, even when the count of viable organisms is  $1.0 \times 10^4$ /ml (e.g. with 3% sodium hydroxide solution at 60°F after 36 hr treatment) the percentage of spores destroyed is 99.99% and thus the percentage still surviving is only 0.01%.

With 3% sodium hydroxide solution at 50°F the time required to produce 99% destruction is approximately 15 hr. If the temperature is reduced by 10°F (to 40°F) the time taken to bring about 99% destruction is trebled, i.e. approximately 44 hr being required. Also, if the concentration of sodium hydroxide is reduced to 2% at 50°F, the time required is doubled, i.e. is approximately 32 hr. When both are reduced, i.e. 2% sodium hydroxide solution at 40°F, the time required is approximately 76 hr, i.e. approximately 5 times that required with 3% sodium hydroxide at 50°F.



## GENERAL CONCLUSIONS

It has been confirmed that Immersion Cleaning is practical and worthwhile with bucket machine milking equipment by the three trials reported in this work.

The results of the spore disinfection studies show that the efficiency of the disinfectant action of sodium hydroxide solution is markedly affected both by concentration and by temperature in the ranges studied. A 2% solution of sodium hydroxide takes twice as long to produce the same degree of disinfection of B. subtilis spores as a 3% solution in the range of temperature 34°F to 82°F. Similarly, reducing the temperature by 10°F in the range 40°F to 70°F approximately doubles the time taken to bring about the same disinfectant effect at all concentrations. Reducing both concentration of sodium hydroxide solution and temperature therefore greatly reduces the efficiency of disinfection.

If the temperature at which the immersion solution is used is low, satisfactory disinfection may not be effected, particularly towards the end of a month's use when the concentration is approaching 2% sodium hydroxide (i.e. 2/3rd of the initial concentration). Therefore, during cold weather it may be necessary either to increase the temperature either of the immersion solution directly or of the milk-house, or to increase the concentration of the sodium hydroxide solution.



The cost of heating and heating equipment may be an uneconomical proposition on some farms. On such farms in Canada, if Immersion Cleaning is used, either the immersion solution must be heated or the concentration of the solution must be increased. Maintaining a 3% solution at 50°F - 60°F produces a slightly better bactericidal effect than using a 5% solution at temperatures just above freezing. But it would be far more economical simply to increase the concentration of sodium hydroxide from 3% to 5% than to heat the immersion solution. Also, one of the advantages of Immersion Cleaning is its simplicity and cheapness. If the equipment was complicated and increased in cost by the inclusion of a heater, and the cost of using Immersion Cleaning considerably increased by having to heat the solution, some of the merits of Immersion Cleaning would be lost.

So, it is proposed that for farms on which the room, where the Immersion Cleaning equipment is housed, is not heated during cold weather, it may be necessary to use an initial concentration of sodium hydroxide of 5% instead of 3% in order to produce a satisfactory disinfectant effect.







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